

Proceedings of the Society for Experimental Biology and Medicine

VOL. 41.

JUNE, 1939.

No. 2.

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Effect of Testosterone Propionate on Behavior of the Female Canary.

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It is well known that marked changes take place in fighting, song, and flocking behavior of birds during the breeding season. In pre-

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vious studies of flock organization the sex of the birds and the state in the reproductive cycle of both sexes played large parts.^{1, 2} In canaries males usually dominated females, except their own mates, at all seasons and both sexes became more aggressive during nesting activity. This domination is manifested by pecking, or a threat to peck, after which the other bird retreats. When birds are first brought together there is usually violent fighting, followed by a more peaceful order when each bird "knows its place."

The sex hormones naturally attracted interest as a possible physical basis for social dominance in the flock. Estrone was injected into female canaries without effect. It was subsequently observed, however, by others^{3, 4, 5} that the ovary produces androgenic substances as well as estrogenic. It, therefore, seemed possible that the male hormone in both sexes was an important factor in social dominance.

In order to test the effect of male hormone, testosterone propionate was administered to 3 of a flock of 6 female canaries. The 3 lowest ranking females, R, T, and C, received .076 mg of testosterone propionate in oil daily. The other 3, H, D, and W, received the same amount of plain oil. In 32 days after the first injection R, C, and T were in 1st, 2nd, and 3rd places, instead of their former places as 4th, 5th and 6th. Fig. 1 shows this shift in dominance.

A detailed record of the changes in pecking order is given in Fig. 2 by the rise and fall of the solid and broken lines. The number of pecks dealt was observed to increase in most cases at the time of the shifting in dominance. More pecks in return indicating unsettled dominance were also seen at this time. There was a shift in dominance in all contact pairs of which one member received hormone and

TABLE I.
Relative Positions of the 6 Female Canaries in the Social Order.

January	February	March
22 28 30	3 8 14 18 20 21 23 25	1 5 9 13 18
D D D	D D D D <i>E E E E</i>	<i>R R R R R</i>
W W W	W W W <i>R T T T T</i>	H H <i>C C C</i>
H H H	H H H W D D D H	<i>T T T T T</i>
<i>C C C</i>	<i>C C C</i> H W W W D	<i>C C</i> H H H
<i>E E E</i>	<i>E E E C</i> H H H C	W W D D D
<i>T T T</i>	<i>T T T T C C C</i> W	D D W W W

Those in italics are birds which began receiving hormone Feb. 5; the others received control injections of oil over the same period.

¹ Allee, W. C., *Wilson Bull.*, 1936, **48**, 145.

² Shoemaker, H. H., *Auk*, **56**, in press.

³ Domm, L. V., *Cold Spring Harbor Symposia on Quant. Biol.*, 1937, **5** 251.

⁴ Witschi, E., and Miller, R. A., *J. Exp. Zool.*, 1938, **79**, 475.

⁵ Noble, G. K., *Anat. Rec.*, 1938, **72**, 60.

the other did not. These results were in accord with those obtained with hens.⁶

On February 14, nine days after injections began, R and C were singing and T began singing regularly March 1. The song was indistinguishable from that of normal males. Just previous to laying females frequently chatter like young males learning to sing and in one case out of about a hundred in these flocks an untreated female uttered a loud song. This was a continued repetition of only one or 2 shrill notes. R and T not only sang an elaborate song but were seen at times singing at each other at full power with heads about

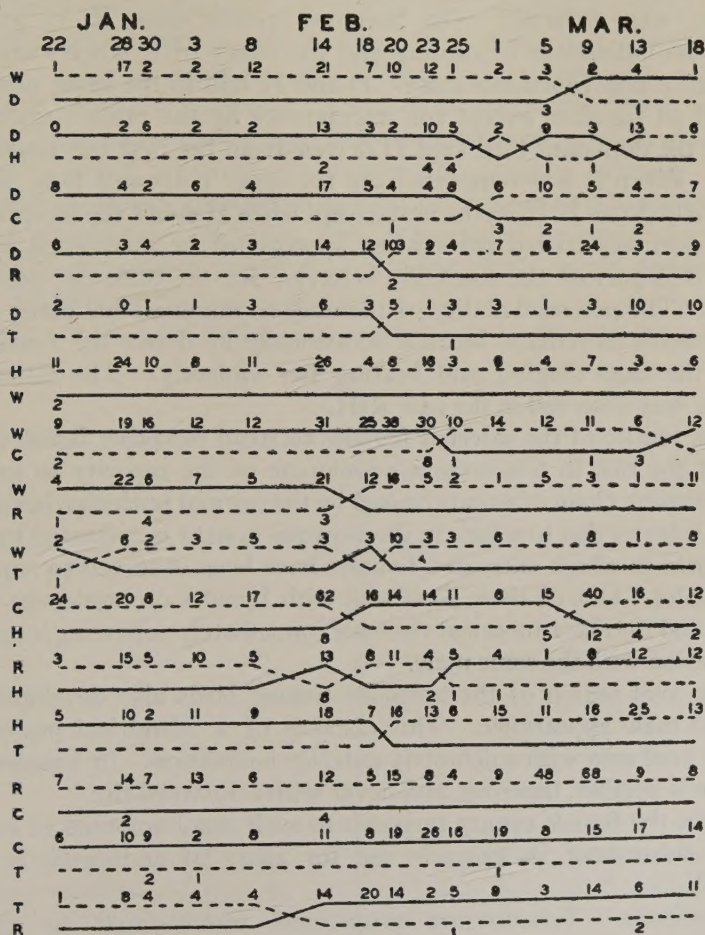


FIG. 1.

Numbers represent pecks delivered on indicated days by birds at left.

⁶ Allee, W. C., and Collias, N., *Anat. Rec.*, 1938, **72**, 60.

an inch apart. This is also typically male behavior, never seen among untreated females. In these injected birds females exhibited courtship behavior characteristic of males, which consisted of a very trim bearing of body with neck outstretched. This behavior often is termed posturing. They also frequently dangled a string or a piece of cotton before other females.

Though copulation was not observed, definite pairing behavior existed between the combinations R-H and T-D. Indications of such also were seen for C-W but not confirmed due to the accidental death of C. H, W, and D all built nests, laid, and incubated but the eggs, of course, were infertile. The hormone treated ones, R, C, and T, made no indications of nesting and the ovary of C when examined showed a non-functional state. D and H laid in the same nest and would sit together except for interference by the other's mate. R being the dominant bird kept D driven from the nest but coaxed H to it. When R was removed from the cage, T allowed D to sit but regularly drove H from the nest except when H could slip in under D.

In normally mated pairs when approached or threatened by his mate it is part of the courtship behavior for the male to "posture" away. The potential ability of the male to dominate his mate, however, seems to remain, because occasionally he drives the female all over the cage singing and beating her violently. The latter behavior was observed in the pair R-H.

The failure of the injected females to tread receptive females may reflect the lack in testosterone propionate of the capacity to initiate the complete chain of events caused by the normal testicular hormone. That it is not due to a lack in the nervous system is indicated by two instances in which untreated females have been observed to copulate like males. One of them copulated with females on numerous occasions and the other mounted the male immediately following a normal copulation with the same male.

The anal region of the hormone-treated birds also developed the typical male appearance. This consists of a cylindrical projection of the anal area with a definitely anterior inclination. In females this region is smaller, tapering, and never with a forward tilt.

Since the female canary responds to such small amounts of androgenic substances, its possible use for assay of androgens is suggested.

Summary. Testosterone propionate administered to female canaries caused suppression of the female reproductive functions and initiation of the following male traits: singing, courtship behavior, peck-dominance over untreated females, and appearance of the male type of anal region.

Quantitative Measurements of Relative Accommodation and Relative Convergence.

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Positive relative accommodation (PRA) is the amount that accommodation can be stimulated toward a point nearer the eyes than that fixated upon, and negative relative accommodation (NRA) the amount accommodation can be stimulated toward a point more distant. PRA is measured by placing before the eyes concave spherical lenses of increasing power until the small detail of the fixated target becomes blurred. NRA is measured by placing before the eyes convex spherical lenses until the detail is blurred. The same basic concepts are applied to relative convergence using prisms in place of spheres. Generally PRC is measured by base-out prisms gradually increased in power until blurring of the target occurs and NRC is measured by base-in prisms.

There are no quantitative data available as to the amount accom-

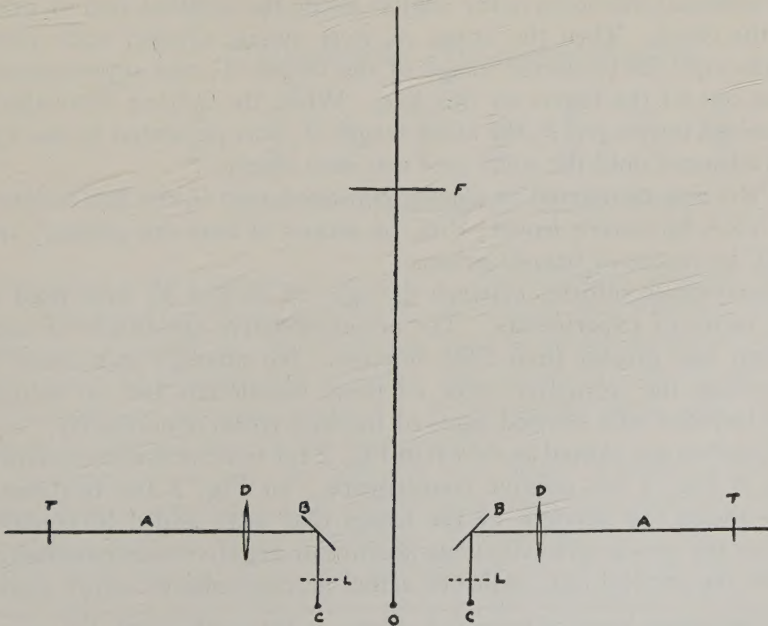


FIG. 1.

modation can be changed without changing convergence and vice versa. The limiting values used by refractionists have been arrived at empirically. The limiting point is said to be reached when the smallest letters on the chart have become too blurred to be read (blur-out).

We have endeavored to arrive at quantitative data by use of an haploscope* which is essentially a mirror stereoscope as shown in Fig. 1. It is made up of two arms *A* that are free to swing around a pivot *C*. Mounted at an angle of 45 degrees to the arms are half-silvered mirrors, *B*. On the arms, *A*, are 2 moveable targets, *T*, whose images are projected to any desired point by lenses, *D*. On these arms are also 2 lens holders, *L*. There is also a central arm on which is another moveable target, *F*, which the subject can see through the half-silvered mirrors. The distance between the pivots, *C*, can be varied to conform to the subject's interpupillary distance.

The targets, *T*, consist of small illuminated pin holes less than 0.2 mm in diameter. Also mounted behind the projection lenses, *D*, are 2 Scheiner discs. Thus when the pin point is conjugate to the retina the subject perceives only one small illuminated spot, but when the point is not conjugate to the retina the subject sees 2 spots.

The target, *F*, was photographically reduced Snellen type. The method used was to have the subject fixate the smallest line of print on the chart. Then the arms, *A*, were swung around their pivot points until the projected image of the target, *T*, was superimposed upon one of the letters of this line. While the subject binocularly perceived the target, *F*, the other target, *T*, was presented to one eye and adjusted until the small spot was seen singly.

PRA was stimulated by placing concave lenses in the lens holders, *L*; NRA by convex lenses; PRC by means of base-out prisms; and NRC by means of base-in prisms.

Forty-three subjects between the ages of 20 and 35 were used in this series of experiments. The accommodative amplitude of each person was greater than 5.00 diopters. No attempt was made to determine the refractive error of these individuals but no subject was included who showed signs of marked visual abnormality.

The data are plotted as shown in Fig. 2 for relative accommodation and in Fig. 3 for relative convergence. In Fig. 2 the horizontal axis shows the strength of the lenses that were added binocularly before the eyes so as to stimulate positive or negative accommodation, while the vertical axis indicates actual accommodative effort as de-

* Courteously loaned to us by F. L. Mason of Physics-Optometry Department of the University of California.

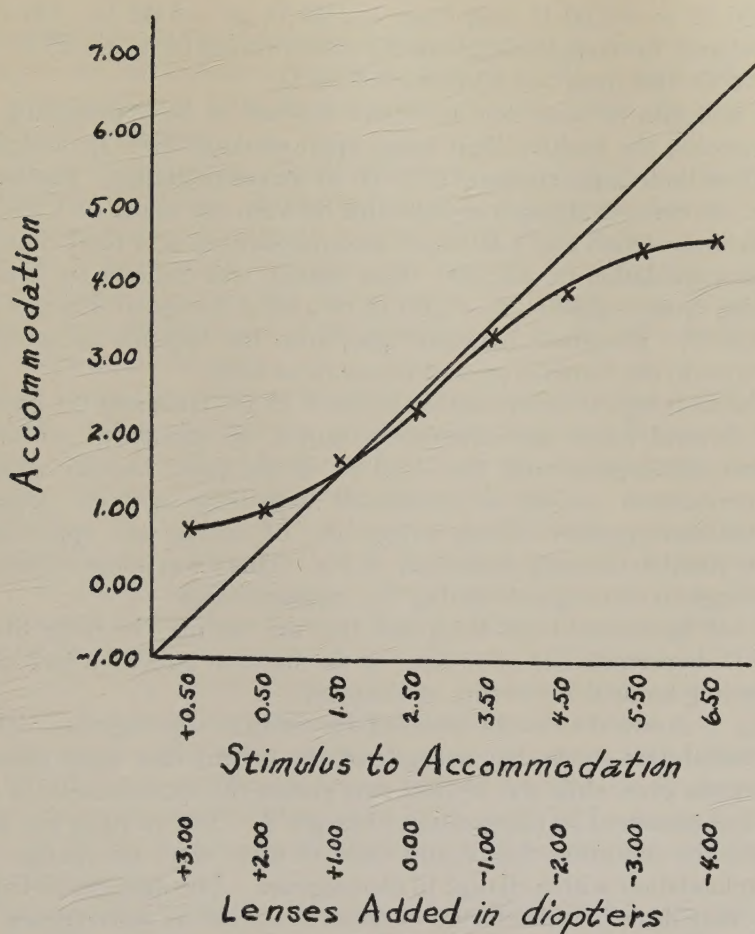


FIG. 2.

terminated by the position of target *T*. The diagonal line represents the theoretical condition based on the assumption that there is a direct one-to-one change in accommodation with the lenses mounted before the eyes. Thus values falling below this line show that the eye was hypermetropic for the conditions of the measurement and values above it indicate that the eye was myopic.

In taking these tests the subjects were asked to report when the small letters were just barely blurred and when they were blurred so that they could no longer read them. The average value obtained for slight blur was +2.00 D. and for blur-out +3.00 D. in NRA; and -3.00 D. for slight blur and -4.00 D. for blur-out in PRA. The greatest variations were for values of slight blur, which ranged from

+1.00 D. to +3.00 D. and from -2.00 D. to -4.00 D. On the other hand, for complete blur-out the values varied from +2.25 D. to +3.00 D. and from -3.50 D. to -5.00 D.

It will also be seen that the curve appears to be approaching 2 asymptotes, the positive limit being approximately 4.75 D. and the negative limit approximately 0.75 D. of accommodation. Furthermore, the curve is almost a straight line between the values of 4.25 D. of accommodation and 1.50 D. of accommodation, or a total change in accommodation of 2.75 D. This change was induced by lenses ranging from -2.50 D. to +1.00 D. or a total change of lens power of 3.50 D. Therefore, between these limits the response of accommodation to the stimulus presented was about 80%.

The most logical interpretation is that 4.25 D. represents the upper limit beyond which accommodation cannot be stimulated without further convergence and that 1.50 D. is the point beyond which accommodation cannot be stimulated negatively without lessening the convergence. These values are, of course, not applicable to any fixation distance other than 40 cm. There was some evidence of change in convergence during the measurements.

It will be noticed from the graph that the eye may be more than 0.75 D. hypermetropic or myopic to the fixation point without experiencing noticeable blurring of the print.

Fig. 3 shows the results obtained for relative convergence. The horizontal axis shows the strength of the prisms that were placed before the eyes while the vertical axis shows the accommodation in force as measured by the position of target *T*. The straight line indicates the condition that would exist if there were no change in accommodation with a change in convergence. The data curve indicates that there is little change in accommodation as convergence is

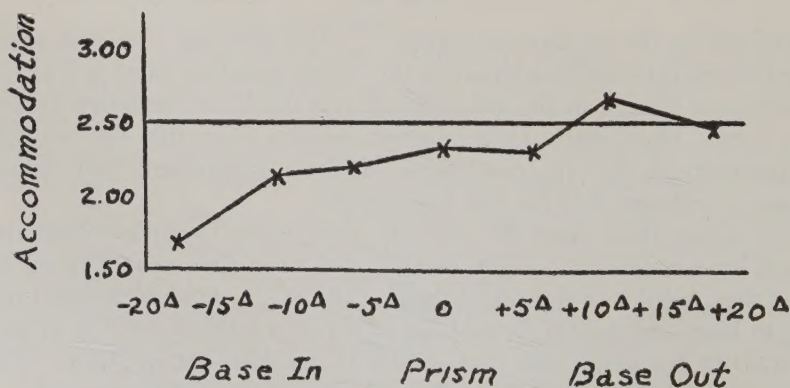


FIG. 3.

increased beyond that needed to fixate the target in the 40 cm plane, but that as convergence is decreased there is a rather sudden change in accommodation between the values of -11 prism diopters and -18 prism diopters.

The average points of blur-out were -14 P.D. for NRC and $+16$ P.D. for PRC. From the graph it is apparent that at -14 P.D. the eye is hypermetropic a little over 0.50 D., and at $+16$ P.D. the eye is emmetropic. This means that the blurring that occurs must be due either to the distortion of the target caused by the prism, or to the tendency of the retinal image to slip off corresponding areas in the retina.

The first explanation can be ruled out as it has been found clinically that the values obtained for positive and negative relative convergence, using a grill of horizontal lines as the fixated target, agree exactly with the values obtained using small type.

From -11 P.D. to $+18$ P.D. there is practically a straight line representation. There is a 0.33 D. change in accommodation with a 29 P.D. change in convergence. For practical purposes it can therefore be said that with each prism diopter change in convergence there is 0.01 D. change in accommodation between the limits of -11 P.D. and $+18$ P.D. relative convergence, assuming that convergence actually changes by the amount represented by the prisms.

Summary. The limits to which accommodation can be changed by interposition of spherical lenses without a change in convergence at a fixation distance of 40 cm are 4.75 D. and 0.75 D. From 4.25 D. to 1.50 D. the actual accommodation is 80% of the lens value used for stimulation.

Convergence cannot be changed without slightly changing accommodation. Between the limits of -11 P.D. and $+18$ P.D. this change amounts to 0.01 D. change in accommodation with each 1.0 P.D. change in convergence.

Developmental Relationship between Epithelial Hypophysis and Infundibulum in *Triturus torosus*.*

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Burch has presented evidence to show that the physiological differentiation of both the anterior and the intermediate lobes of the pituitary requires the contact of the epithelial hypophysis with nervous tissue, specifically the floor of the infundibulum.^{1, 2} In experimental embryos of *Hyla regilla*, contact of hypophysis with infundibulum was prevented by early translocation of the infundibular anlage to a position in the hind brain. Such embryos developed into silvery-white tadpoles which failed to metamorphose. The "albinism" was shown to be caused by a lack of secretion of the *pars intermedia*, whereas the failure to metamorphose was attributed to lack of thyrotropic principle of the anterior lobe. Histological examination showed no evidence of differentiation in the hypophysis, a mass of deeply staining material occupying its normal position at the tip of the notochord, yet some distance away from the transplanted infundibulum. It was suggested by Burch that the infundibulum acts as an organizer upon the epithelial hypophysis.

I undertook experiments upon a urodele, *Triturus torosus*, to confirm Burch's findings. Using a method described earlier,^{3, 4} gelatin was injected into 2 series of embryos in early tail-bud stage, namely, into the cavities of the brain of one series and into the foregut of the other. In the former series the ventricles of the brain, including the infundibular recess, were inordinately distended. In several instances the epithelial hypophysis failed to reach the floor of the infundibulum in its migration inward from the stomodeum, probably because of pressure exerted by the gelatin within the brain. These tadpoles exhibited without exception the syndrome of "albinism." The melanin in both epidermal and dermal melanophores was highly concentrated, whereas the pigment within the xantholeucophores was markedly dispersed. Histological examination disclosed the hypophysis as a mass of undifferentiated cells lying beneath the dien-

* Aided by Public Works Administration, Official Project No. 465-03-3-192.

1 Burch, A. B., *Proc. Soc. Exp. Biol. and Med.*, 1938, **38**, 608.

2 Burch, A. B., *Ibid.*, 1939, **40**, 341.

3 Eakin, R. M., *Univ. Calif. Publ. Zool.*, 1933, **39**, 191.

4 Eakin, R. M., *Ibid.*, 1938, **43**, 185.

cephalon and immediately anterior to the infundibular recess. As the larvae were sacrificed at the end of 5 weeks information regarding metamorphosis is not available.

In the second series, in which the foregut was maximally distended with gelatin, the hypophyseal anlage was recognizable at the time of injection as a patch of whitish cells in the region of the future stomodeum. In some specimens this cluster of cells remained entirely outside for 5 or 6 days after injection. As a result either of the enlargement of the buccal cavity through growth or of the partial liquefaction of the gelatin, the hypophysis was permitted eventually to migrate inward.

All larvae which were successfully maintained for 6 weeks showed normal pigmentation, except one. This one specimen exhibited the condition of "albinism" characteristic of hypophysectomized tadpoles of *Triturus*⁵ and similar in all respects to the syndrome observed in the first series. Microscopic sections of the specimen showed the hypophysis as a block of undifferentiated material lying between the optic recess and the roof of the pharynx. In one place the cells of the hypophysis still formed a part of the pharyngeal epithelium. The gelatin had apparently prevented the epithelial hypophysis from reaching its normal position at the base of the infundibulum. Furthermore, large masses of cartilage, which evidently developed in response to the pressure exerted by the mass of gelatin within the foregut, imprisoned the hypophysis, thus precluding all possibility of its ever completing its inward migration. Although in contact dorsally with the optic recess, the hypophysis apparently induced no hyperplasia in the nervous tissue, as noted by other investigators.^{1, 6}

The experimental larvae, other than the "albino" described above, were indistinguishable from the controls as regards pigmentation. Microscopic sections of each revealed, however, that the experimental procedure had not completely prevented the epithelial hypophysis from establishing contact with the infundibulum. In some instances it had succeeded in migrating to its normal definitive position at the posterior limit of the infundibular recess; in others it came to lie along the antero-ventral wall of the infundibulum. In one instance just a few posteriormost cells of the hypophysis reached infundibular tissue; for the most part the hypophyseal cells extended forward and ventrad through a foramen in the chondrocranium. Yet apparently this one point of contact was sufficient for the functional differentiation of the *pars intermedia*, at least. Since the larvae

⁵ Miller, A. H., unpublished manuscript.

⁶ Smith, P. E., *Am. Anat. Mem.*, **11**

of this series likewise were fixed prior to the time of metamorphosis, no information upon the functional differentiation of the anterior lobe is available.

Although the number of "albinos" in each series is very low, the evidence is so unmistakable that one may safely conclude that the developmental relationship between the epithelial hypophysis and infundibulum in urodeles is essentially as Burch has shown for *Hyla*, namely, that the functional differentiation of the former is dependent upon contact with the latter.

10655

Studies on the Cause of Increased Growth During Pregnancy.

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Slonaker,¹ Hain,² and Stein³ have shown that pregnancy favorably affected the growth curve in rats over littermate virgin controls. Cole⁴ observed that even in pregnant rats precociously matured with mare gonadotropic hormone there was an increased growth rate and this was confirmed by Cole and Hart.⁵ The increased rate of growth was accompanied by increased food consumption starting as early as 48 hours after copulation. Lactation neither enhanced nor reduced this favorable effect. The data in this paper relate to the factors involved in this phenomenon.

Pseudopregnancy. Goss⁶ and Slonaker⁷ showed that a single pseudopregnancy increased growth in the rat though the effect of repeated pseudopregnancies was not determined. In our previous paper⁵ one animal was cited having only 2 pregnancies followed by 13 periods of pseudopregnancy over a 9-month period. This animal continued to gain over her littermate control throughout this period and at autopsy this increase amounted to 119 g. We were, therefore, interested to ascertain if repeated pseudopregnancies would have as marked an effect as normal pregnancies had shown. This is im-

¹ Slonaker, J. R., *Am. J. Physiol.*, 1927, **82**, 318.

² Hain, A. M., *Quart. J. Exp. Physiol.*, 1932, **22**, 71.

³ Stein, S., *Endocrinology*, 1934, **18**, 721.

⁴ Cole, H. H., *Am. J. Physiol.*, 1937, **119**, 704.

⁵ Cole, H. H., and Hart, G. H., *Am. J. Physiol.*, 1938, **123**, 589.

⁶ Goss, H., *Anat. Rec.*, 1926, **32**, 232.

⁷ Slonaker, J. R., *Am. J. Physiol.*, 1929, **89**, 406.

portant if true because it rules out the fetus and fetal placenta as a factor in its causation.

In Fig. 1 are shown relative average growth rates of 5 rats carried through an average of 20 pseudopregnancies as compared to littermate virgin controls. Also included are the average growth curves of 5 rats carried through 9 normal pregnancies.

This figure shows that the increased growth response from repeated pseudopregnancies is nearly as great as that obtained from a series of true pregnancies. The average nose-anus length of the pseudopregnant rats was 23.0 as compared to 22.2 cm for the controls.

Inasmuch as only 5 pseudopregnant animals were used it seemed desirable to run another larger group. Ten others were carried through 6 pseudopregnancies and compared to littermate virgin controls. These rats were carefully selected for size in order that there would be a minimum of variation between and within the groups. At 140 days this group averaged 307 g in weight and 22.0 cm in

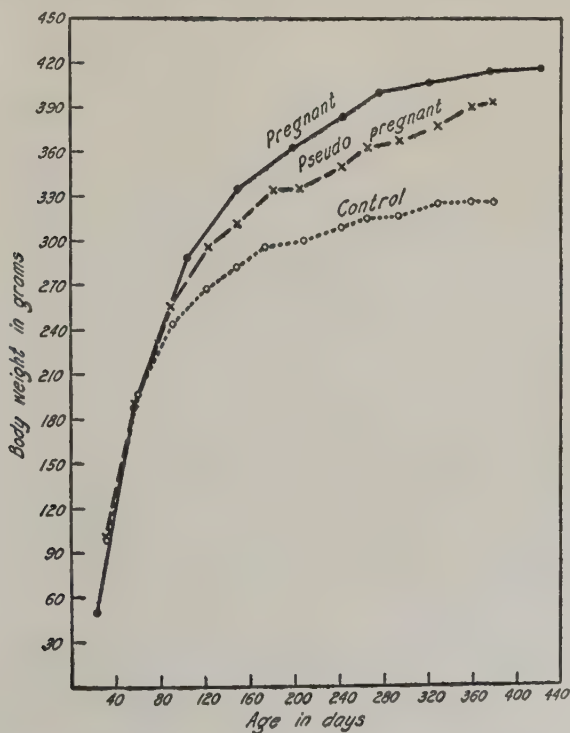


FIG. 1.

The growth rate of 5 rats carried through an average of 20 pseudopregnancies as compared to that of 5 littermate virgin controls and to that of 5 rats carried through 9 normal pregnancies.

nose-anus length as compared to 257 g and 20.8 cm for their controls—a difference of 50 g and 1.2 cm. As may be seen from Fig. 1 the original pseudopregnant group weighed 305 g and their controls 275 g at this age—a difference of 30 g. As the increase of the second pseudopregnant group over their controls exceeded that of the first at this age we felt it was unnecessary to carry them further.

The fact that repeated pseudopregnancies have nearly as marked an effect on growth as true pregnancies makes it appear that the fetuses and their attending membranes are not responsible for stimulating the increased growth during pregnancy. We, therefore, looked for other causes.

Effect of the gonads. Two approaches were made in the study of the question. First we compared the rate of growth of 9 rats, castrated 24 hours after mating with normal males, to the rate of growth of 9 littermate controls castrated on the same day without previous mating. This experiment was staged to ascertain if increased food intake of the bred animals would occur as rapidly in the absence of the ovaries.

The very definite anticipated increase in appetite within 48 hours after mating did not occur. This delay might possibly be laid to the operation. The surgery did reduce appetite during the first 24 hours but by the second day it had returned to the preoperation level. As an increased appetite occurred in both groups on the 6th day it is unlikely that mating had any effect in the absence of the ovary. This inference that the ovary might be involved in stimulating appetite caused us to study the effect of progesterone on this phenomenon. Gaunt and Hays⁸ have shown that progesterone has an influence on appetite in the adrenalectomized animal.

First, attempts were made to maintain pregnancy in rats castrated 24 hours after mating by use of progesterone,* but the doses used were insufficient. The 7 animals receiving progesterone for 20 days after castration had an average daily food consumption of 15.3 g while their littermate controls castrated but receiving no progesterone had an average daily food consumption over the same period of 17.6 g.

A second experiment using progesterone was then carried out. In this case groups of 4 normal virgin females were used with littermates distributed between the groups. For 12 days one group received 1.5 mg daily of progesterone dissolved in sesame oil, followed by

⁸ Gaunt, R., and Hays, H. W., *Am. J. Physiol.*, 1938, **124**, 767.

* Progesterone (Proluton) was supplied through the courtesy of Dr. Erwin Schwenk, Schering Corporation, Bloomfield, N. J.

3 mg doses daily for 8 additional days. Another group received sesame oil alone. The dose was increased because 1.5 mg proved to be insufficient to stop the cycle while 3 mg did this successfully. The average weight of the progesterone group at the beginning and end of the injections was 179 g and 223 g respectively, and for the controls 180 g and 213 g respectively. The average daily food consumption for the 2 groups was 12.7 g for the progesterone group and 13.0 g for the sesame controls. These experiments give no evidence that progesterone stimulates appetite.

Conclusions. 1. Inasmuch as pseudopregnant rats gain at nearly the same rate as those going through normal pregnancy it would appear that the fetus and its membranes are responsible only in part, if at all, for the increased growth rate of pregnant rats. 2. Progesterone apparently does not stimulate appetite. If the ovary is indirectly involved it may be by depressed estrogen secretion following mating inasmuch as appetite is increased following castration. This increase, however, is not observed until the sixth day as compared to the second day in pregnancy.

10656

Gonadotropic Hormones. VII. Influence of length of period of administration of equine hormone.*

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In a previous study¹ it was shown that the administration of a *known total dose* of chorionic gonadotropic hormone prepared from blood of pregnant women over periods of 10, 15, or 20 days, produced a much greater increase in ovarian and uterine weights of immature rats than when the same total dose was given in 5 days (Fig. 1). The opposite result was obtained with an acid extract of sheep anterior pituitary glands, as the injection of a known dose in 5 days produced a greater increase in ovarian weight than when the administration of the same total dose was spread out over periods of 10, 15,

*Supported in part by a grant from the Committee for Research in Problems of Sex, National Research Council. The gonadin was kindly supplied by the Cutter Laboratories.

¹ Fluhmann, C. F., *Proc. Soc. Exp. Biol. and Med.*, 1933, **30**, 1014.

or 20 days (Fig. 1). This report deals with a study of this "time factor" in the case of equine gonadotropic hormone.

The preparation employed is an extract of pregnant mares' serum made by the Cutter Laboratories and named Gonadin. It was made up in various dilutions so that each animal received a total of 28 rat units (Cutter) when 0.25 cc was administered twice daily for the duration of the experiment. The injections were begun when the rats were 21 to 23 days of age, and a total of 20 animals was employed.

The results indicate that this hormone acts differently from either the chorionic or the gonadotropic factors, since the increase in ovarian weight was about the same irrespective of the length of the period of injection, while there was a progressive increase in the weight of the uterus (Fig. 1). Six rats injected for 5 days gave an average body weight of 38 g, ovaries .039 g, and uterus .128 g. The same total dose given in 10 days to 7 rats resulted in an average body weight of 47 g, ovaries .031 g, and uterus .109 g. In 15 days the average figures for 7 rats gave body weight 64 g, ovaries .033 g, and uterus .238 g.

Summary. The administration over various periods of time of a known total dose of an extract of equine gonadotropic hormone to immature rats gave results different from those obtained with either sheep anterior lobe or chorionic gonadotropic hormones. Ovaries of about the same weight were obtained whether the injections were carried out for 5, 10, or 15 days, but as the period of injection was prolonged there was a progressive increase in uterine weight.

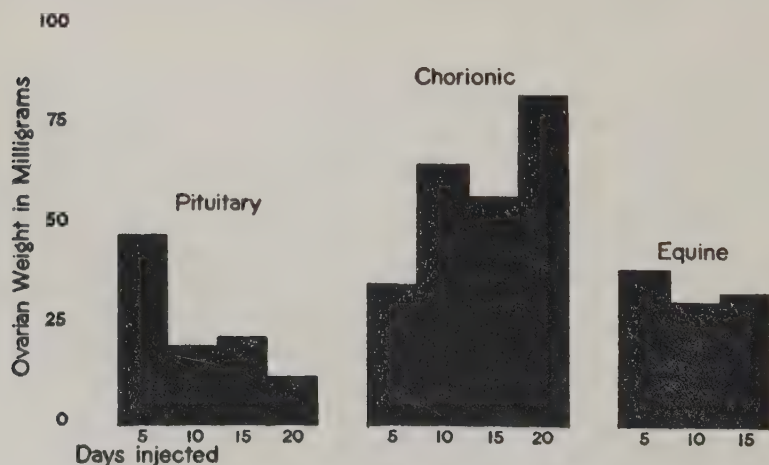


FIG. 1.

10657

Reduced Muscle Creatine in Paralyzed Young E-Low Rats.*

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*From the Institute of Experimental Biology and the Division of Anatomy, School
 of Medicine, University of California, Berkeley.*

Olcott¹ first observed that muscular dystrophy occurred in the suckling young of vitamin E-low rats. Knowlton and Hines² noted a slight drop in muscle creatine in 4- to 6-months-old E-deficient rats with no visible signs of paralysis. Goettsch and Brown³ demonstrated a loss in skeletal muscle creatine in rabbits suffering from nutritional muscular dystrophy. Later Ni,⁴ using the same diet, noted in guinea pigs a corresponding creatine loss in the skeletal musculature accompanying the muscle degeneration. Morgulis⁵ reported a marked increase of creatine in the urine of rabbits held on a dystrophic diet. These findings suggested the probability that more significant changes in muscle creatine would be found in suckling young rats when extensive paralysis was evident.

Experimental. Each member of a group of young E-low females with a history of a resorption gestation was given a single dose of 1.0 g of wheat germ oil on the day following copulation. The litters resulting from these pregnancies were reduced to 6 young each. On the day of parturition one-half of the mothers were given 2.0 g of wheat germ oil by stomach tube. All mothers received Diet 808.†

* Aided by grants from the Board of Research and the Department of Agriculture of the University of California, from Merck and Company, Inc., and from the Rockefeller Foundation, New York. Assistance was rendered by the Federal Works Progress Administration, Project 8877 A-5. The following materials were generously contributed: brewers' yeast by The Vitamin Food Company of New York, cod liver oil by E. R. Squibb and Sons, and wheat germ from which oil was prepared by General Mills, Inc.

¹ Olcott, H. S., *J. Nutr.*, 1938, **15**, 221.

² Knowlton, G. C., and Hines, H. M., *PROC. SOC. EXP. BIOL. AND MED.*, 1938, **38**, 665.

³ Goettsch, M., and Brown, E. F., *J. Biol. Chem.*, 1932, **97**, 549.

⁴ Ni, T. G., *Chinese J. Physiol.*, 1936, **10**, 199.

⁵ Morgulis, S., *Nutritional Muscular Dystrophy*, Paris, Hermann and Cie, 1938.

† Diet 808:	casein (commercial)	27
	cornstarch (cooked)	30
	lard	22
	cod liver oil	2
	brewers' yeast	15
	salts No. 185	4

(Ingredients except cod liver oil were allowed to stand for 2 weeks at room temperature in order that the rancidity of the lard would destroy the vitamin E in the diet. The cod liver oil was added just before feeding.)

One-half of the members of each litter were transferred to a foster mother (3 of the young of the mother destined to receive wheat germ oil were given to an untreated mother, and vice versa). At the end of the lactation period the young suckled by the treated mothers appeared normal and served as controls. Half of each group were sacrificed on day 21; the remainder were sacrificed on day 45. The fresh muscle creatine content of the gastrocnemius and soleus muscles was determined by the Rose-Helmer-Chanutin technic⁶ (Table I).

Parts of these same muscles were saved for microscopic examination. There was a marked drop in the muscle creatine in the E-low animals as contrasted with that in the normal group (average values 152 mg and 242 mg respectively). This loss of 37% of the creatine content was paralleled by extensive muscle degeneration. The paralyzed young which were allowed to survive until the 45th day recovered spontaneously although in all cases the initial paralysis was of the same severity as that affecting their littermates sacrificed at day 21. When autopsied they presented the outward appearance of normal rats. The skeletal muscle creatine was determined and found to be normal, average values being 245 mg as compared with 246 mg for the control group. Histologically, also, the muscles of these rats appeared normal (paper in press). Thus a regeneration of muscle elements had occurred spontaneously without the addition of any vitamin E to the diet.

Since the animals in the E-low group in the first part of this experiment had just been weaned and were partially or completely paralyzed, they may have found it difficult to procure a sustaining quantity of diet. Hence the sudden change in muscle creatine might have been due to a semi-starvation, as suggested by studies on starvation by Mendel and Rose⁷ and Myers and Fine.⁸ The following experiment was devised to check the possible influence of starvation upon muscle creatine. Three litters, a total of 16 young, from normal mothers on stock diet, were used. Nine young remained with their mothers continuously throughout the period of the experiment. The remaining 7 rats were subjected to a semi-starvation regimen from day 5 to day 21. This regimen consisted of removing the young from their mothers 8 hours per day from day 5 to day 10; 12 hours daily from day 11 to day 15; 16 hours daily from day 16 to day 21. These animals were kept in a well ventilated incubator held at 37°C. The

⁶ Rose, W. C., Helmer, O. M., and Chanutin, A., *J. Biol. Chem.*, 1927, **75**, 543.

⁷ Mendel, L. B., and Rose, W. C., *J. Biol. Chem.*, 1911-12, **10**, 255.

⁸ Myers, V. C., and Fine, M. S., *J. Biol. Chem.*, 1913, **15**, 283.

TABLE I.
Skeletal Muscle Creatine Values in Young E-low Rats.

	No. of animals	Age at autopsy, days	Body wt at autopsy, g	Previous history	Condition at autopsy	Avg creatine value mg/100 g tissue
Diet 808						
Mother given 2.0 g wheat germ oil	10	21	41	normal	normal	242
Diet 808						
Mother E-low	9	21	38	"	paralyzed	152
Diet 808						
Mother given 2.0 g wheat germ oil	4	45	115	"	normal	246
Diet 808						
Mother E-low	5	45	108	paralyzed from day 21-25	normal (spontaneous recovery)	245
Stock diet	9	21	54	normal	normal	218
Stock diet (semi-starvation)	7	21	37	semi-starvation from day 5-21	undernourished	221

rats were given water by stomach tube once every 4 hours during the early stages of their incubator life, in order to prevent excessive dehydration. After day 15 this treatment was unnecessary. The average weaning weight of the experimental group was 37 g as compared with the littermate control group with 54 g. The 2 average creatine values are practically identical, being 221 mg and 218 mg respectively. It would appear from this study that semi-starvation for the last 16 days of lactation does not create a loss in muscle creatine. Therefore the drop in creatine value in the E-low paralyzed group was not occasioned or influenced by a semi-starvation which might conceivably have occurred.

Summary. A marked loss in skeletal muscle creatine occurs during the paralysis of suckling rats from mothers held on an E-low diet. These muscles showed extensive degeneration. There was a subsequent rise to normal creatine values with spontaneous recovery from the paralysis. Such muscles showed histological normality. The lowered muscle creatine was not produced by inanition.

10658

Failure to Produce Abdominal Neoplasms in Rats Receiving Wheat Germ Oil Extracted in Various Ways.*

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Rowntree and coworkers¹⁻⁴ reported the production of abdominal neoplasms in rats of the Wistar, Buffalo and Yale albino strains held for varying periods and on different intake of a crude ether extracted

* Aided by grants from the Research Board and the College of Agriculture of the University of California and the Rockefeller Foundation of New York City, and from Merck and Company, Rahway, New Jersey. We wish to acknowledge the assistance rendered by the Federal Works Progress Administration (Project No. 10482-85). The following materials were generously supplied: Cod liver oil by E. R. Squibb and Sons; wheat germ, pressed wheat germ oil, and wheat germ oil concentrate by General Mills, Inc.; brewers' yeast by Vegex, Inc.

¹ Rowntree, L. G., Lansbury, J., and Steinberg, A., *PROC. SOC. EXP. BIOL. AND MED.*, 1937, **36**, 424.

² Dorrance, G. M., and Ciccone, E. F., *PROC. SOC. EXP. BIOL. AND MED.*, 1937, **36**, 427.

³ Rowntree, L. G., Steinberg, A., Dorrance, G. M., and Ciccone, E. F., *Am. J. Cancer*, 1937, **31**, 359.

⁴ Rowntree, L. G., Steinberg, A., and Brown, W. R., *Trans. Assn. Am. Phys.*, 1938, **53**, 199.

wheat germ oil. In the Wistar strain tumors were palpable as early as 15 days (and in all animals by 111 days) following the administration of 4 cc of wheat germ oil. Carruthers⁵ in Mattill's laboratory, employing albino rats of the Wistar and Sprague Dawley strains, was unable to confirm Rowntree's findings. Day, Becker and McCollum⁶ were unable to show that ether *per se* played a part as they failed to produce neoplasms in the McCollum strain when they fed a pressed wheat germ oil that had been refluxed with peroxide-free ether and a faint trace of ether remained in the wheat germ oil.

In connection with other studies carried out in this laboratory, rats of the Long-Evans strain have been maintained for long periods on various intakes of a crude wheat germ oil prepared by extracting the fresh germs with a high grade petroleum ether. It was impossible to free the oil of residual traces of solvent. This oil was at first clear but a sediment (probably sterols) separated on standing. A wheat germ oil so prepared was active as vitamin E in a single dose of 0.5 g when administered to vitamin E-low females of proved sterility. The experiments, in which the pressed and ether-extracted wheat germ oils were fed, were planned to test the Rowntree findings.

The ether extracted wheat germ oil was prepared exactly according to the method used by E. R. Squibb and Sons, who furnished the oil used by Rowntree. The cold pressed wheat germ oil was assayed for vitamin E activity and found potent in a single dose of 500 mg and the concentrate at 100 mg.

The wheat-germ-oil-containing diets employed were as follows:

	791	789	798	809
Casein (commercial)	27	27	34	27
Cornstarch (cooked)	35	35	—	27
Salts (McCollum 185)	4	4	4	4
Lard	12	—	—	—
Wheat germ oil†	10	22	50	30
Cod liver oil	2	2	2	2
Brewers' yeast	10	10	10	10

All diets were made in sufficient quantity for one week's feedings and stored at 0°C. They were fed daily in porcelain containers. The 100 mg of the concentrate from wheat germ oil was fed by dropper daily. The animals in this group were maintained on our standard E-low diet No. 427⁷ as were those receiving the supplement of 2 cc of wheat germ oil.

⁵ Carruthers, C., *PROC. SOC. EXP. BIOL. AND MED.*, 1939, **40**, 107.

⁶ Day, H. G., Becker, J. E., and McCollum, E. V., *PROC. SOC. EXP. BIOL. AND MED.*, 1939, **40**, 21.

† Wheat germ oil extracted with high grade petroleum ether, Diets 791 and 789; wheat germ oil extracted with ether, Diet 809; wheat germ oil pressed, Diet, 798.

⁷ Emerson, G. A., and Evans, H. M., *J. Nutr.*, 1937, **14**, 169.

TABLE I.
Wheat Germ Oil Experiments. Autopsy Findings Normal.

No. of rats	Sex	Wheat germ oil			Days fed
		Type	Quantity fed 6 times weekly	As % of diet ad lib.	
20	F	petroleum ether extracted	2 cc		45
13	F	" " "		22 (Diet 789)	45
14	F	" " "		10 (" 791)	365
14	F	" " "	4 drops		365
5	M	" " "	4 "		277
12	F	pressed		50 (" 798)	180
6	M	" "		50 (" 798)	180
6	F	vitamin E concentrate	100 mg		180
3	M	" " "	100 mg		180
6	F	ether extracted		30 (" 809)	370
2	M	ether extracted		30 (" 809)	370

The animals in all groups were autopsied at the end of the periods indicated (45-370 days) and a careful examination of all organs was made. In no case was a neoplasm found (Table I) even in the case of rats maintained on the 30% ether extracted wheat germ oil for a period of 370 days. It must be pointed out that our rats are not of the albino strain and may be sturdier than those employed by Rowntree.

Conclusion. Rats of the Long-Evans strain did not develop abdominal neoplasms when maintained for periods from 45-370 days on high wheat-germ-oil-containing rations. One hundred and one animals were employed in these experiments.

10659 P

Blood-CNS Barrier Permeability to Horse Serum In Experimental Poliomyelitis.*

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It is generally accepted that the passage of foreign substances between the blood and spinal fluid is controlled by a physiologic barrier interposed between the central nervous system and other,

* This work was supported by a grant from the National Foundation for Infantile Paralysis to Washington University.

non-nervous tissues. This barrier, usually referred to as the blood-brain (or blood-CNS¹) barrier, is regarded as a functional entity² composed of various structures of the cerebrospinal nervous system. Stern and her collaborators³ have adduced evidence that certain portions of the barrier are concerned mainly with the passage of crystalloids while other structures are concerned with colloidal substances.

We have previously reported that the pathologic changes occurring in the CNS of *Rhesus* monkeys infected with poliomyelitis virus increase the permeability of the barrier to crystalloids such as sodium nitrate⁴ and sodium bromide.⁵ Since the penetration of crystalloids and colloids from the blood into the cerebrospinal fluid appears to be controlled by 2 different mechanisms, we believed the bearing of this subject on chemotherapy and serum therapy warranted further examination. While this work was in progress, Kempf, Nungester and Soule⁶ reported that rabbit antisheep hemolysin passes the barrier in insignificant amounts; this experience agrees well with that of Shaughnessy, Grubb and Harmon,⁷ who used several antibody-containing sera.

In these experiments we have used *Rhesus* monkeys in various stages of the disease following cerebral or nasal inoculation of the potent MV strain of virus. In order to maintain during the experiment approximately constant concentration of foreign protein in the bloodstream, normal horse serum† was administered subcutaneously in 5.0 cc amounts twice daily for 2 consecutive days. On the 3rd day, specimens of blood and spinal fluid for analysis were obtained by cardiac and cisternal puncture under deep ether anesthesia. Detection and rough estimation of the concentration of horse serum in the serum and spinal fluid of monkeys was accomplished by using standardized precipitating serum from rabbits which had been immunized with horse serum. The concentration of horse serum present was calculated from the "end-point" or limiting dilution

¹ Lennette, E. H., and Hudson, N. P., *PROC. SOC. EXP. BIOL. AND MED.*, 1936, **34**, 470.

² Katzenelbogen, S., *The cerebrospinal fluid and its relation to the blood*, Baltimore, Johns Hopkins Press, 1935.

³ Stern, L., quoted from Katzenelbogen, *loc. cit.*, p. 83.

⁴ Lennette, E. H., and Reames, H. R., *J. Immunol.*, 1938, **34**, 215.

⁵ Lennette, E. H., and Campbell, D. H., *Am. J. Dis. Child.*, 1938, **56**, 756.

⁶ Kempf, J. E., Nungester, W. J., and Soule, M. H., *PROC. SOC. EXP. BIOL. AND MED.*, 1939, **40**, 395.

⁷ Shaughnessy, H. J., Grubb, T. C., and Harmon, P. H., *J. Bact.*, 1937, **32**, 47.

† We are indebted to Parke, Davis and Co. for a generous supply of normal horse serum.

required to form a precipitate with the previously standardized antiserum. For example, if the limiting dilution of a sample of monkey serum containing horse serum was 1:600 when tested against antiserum having a titer of 1:100,000, the concentration of horse serum was expressed as $600/100,000 = 0.600\%$. The antiserum was standardized by titration against a known amount of horse serum added to monkey serum or spinal fluid. When testing an unknown specimen, a preliminary titration was first done to ascertain the approximate range and then a second titration was performed to secure a more accurate value. The final dilutions of spinal fluid tested were 1:2, 1:4, 1:6, 1:8, etc., and of serum 1:50 with each succeeding dilution increased by 20%. Although a few of the tests were carried out by the use of the "ring" method, the majority were done by mixing 0.3 cc amounts of the antiserum which had been diluted with one volume of 0.5% saline, with 0.3 cc of the test solution in small thin-walled tubes. The mixtures were incubated for 1 hour at 45°C, then placed in a refrigerator at 4°C, and read after 2 hours.

Since we⁸ among others have noted that determination of the concentration of the test substance in the spinal fluid alone gives only a very rough idea of barrier permeability, the results are expressed here as a ratio,

$$\frac{\text{horse serum in blood}}{\text{horse serum in spinal fluid}}$$

which is referred to as the P.Q. (Permeability Quotient). The P.Q. values given in Table I represent the following ratio:

$$\frac{X_s/S}{X_{csf}/S}$$

where X_s represents the limiting dilution of the unknown sample of monkey serum, X_{csf} the limiting dilution of spinal fluid and S the titration value of the standard antiserum. This procedure gives a more precise idea of barrier permeability, and it should be noted that a high P.Q. value corresponds to a low degree of permeability, and that as permeability increases, the P.Q. value decreases.

From Table I it will be seen that we were unable to detect any horse serum in the spinal fluid of normal control monkeys and hence the P.Q. could not be calculated. On the other hand, there was some increased permeability to horse serum in the infected animals. The quotients obtained were much higher than those obtained with sodium bromide⁵ and tend to approximate those obtained by Bur-

⁸ Lennette, E. H., Campbell, D. H., and Reames, H. R., *PROC. SOC. EXP. BIOL. AND MED.*, 1939, **40**, 287.

TABLE I.
Permeability of Blood-CNS Barrier to Horse Serum in Poliomyelitis.

History of monkeys	No. of monkeys	Permeability quotients
Normals	5	0*, 0, 0, 0, 0
Preparalytic	3† 4‡	100, 120, 200 20, 40, 160, 200
Paralytic	4† 4‡	160, 240, 340, 760 20, 60, 120, 600

* No horse serum detected in spinal fluid.

† Poliomyelitis following nasal instillation of virus.

‡ " " " " intracerebral inoculation of virus.

tenshaw⁹ in human meningococcus meningitis. The high P.Q. values indicate that horse serum penetrated the barrier in small and, from a therapeutic standpoint, probably insignificant amounts. This may be due in part to the fact that horse serum disappears from the bloodstream relatively rapidly and hence fails to maintain the concentration necessary for penetration of the material into the spinal fluid. Whether the same situation obtains with homologous protein is now under study.

Summary. Normal horse serum administered in the quantity and manner described was found to penetrate the blood-CNS barrier of poliomyelitic animals regularly although in small amounts. From a therapeutic standpoint, it would seem that under the stated conditions such concentrations of serum would probably be inadequate.

10660

Comparison of Activity of Viruses of St. Louis and Japanese Encephalitis in the Chick Embryo.

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The close similarity in the clinical and pathologic pictures of the St. Louis and the Japanese Type B encephalitis in both man and the mouse suggested a comparative study of the activity of these viruses in the chick embryo.

⁹ Burtenshaw, J. M. L., *Lancet*, 1938, **2**, 1513.

The St. Louis encephalitis virus has been grown^{1,2} and passed for long periods³ on the chorioallantoic membrane of the developing chick. Haagen and Crodel⁴ have recently reported the cultivation and prolonged passage of the Japanese encephalitis virus on this medium. In the present experiments a comparison was made of the anatomical changes produced by the 2 viruses and of the degree of multiplication which took place in the egg in the 2 instances. In each series of experiments the original inoculum of the chorioallantoic membrane consisted of 0.05 cc of the supernatant fluid from a 10% broth suspension of mouse brain infected with the respective virus. Eggs incubated from 10 to 13 days were used. The virus of St. Louis encephalitis was passed in series for a number of months and the virus of Japanese encephalitis* was carried through 7 passages in the egg. With the St. Louis virus, prolonged cultivation did not alter the original pathologic picture seen in the earlier passages.

At 3 and 4 days after inoculation the appearance of the chorioallantoic membranes inoculated with the Japanese encephalitis virus is grossly indistinguishable from that of eggs inoculated with the St. Louis virus. The most conspicuous finding is the edema of the membranes. In both cases the membranes are slightly opaque, and a very fine stippling is apparent. At 5 days the membranes inoculated with either virus are uniformly opaque and the degree of edema is less. A somewhat greater tendency to the formation of necrotic foci is observed with the Japanese encephalitis virus. However, necrotic foci may occur with either virus, most frequently where the membranes have been traumatized. Membranes inoculated with either virus present a similar microscopic picture. The same sort of diffuse proliferation of the ectoderm with focal accentuations of this process occurs in each case. Vacuolization and necrosis of the surface layers of the ectoderm take place especially in areas where the proliferation is accentuated. For the most part the deeper layers of the ectoderm remain intact but at times it becomes completely necrotic, leaving denuded surfaces corresponding to the occasional gross ulcerations. Downgrowths of the ectoderm into the mesoderm also occur.

¹ Harrison, R. W., and Moore, E., *Proc. Soc. Exp. Biol. and Med.*, 1936, **35**, 359; *Am. J. Path.*, 1937, **13**, 361.

² Schultz, E. W., Williams, G. F., and Hetherington, A., *Proc. Soc. Exp. Biol. and Med.*, 1938, **38**, 799.

³ Smith, Margaret G., *Proc. Soc. Exp. Biol. and Med.*, 1939, **40**, 191.

⁴ Haagen, E., and Crodel, B., *Zentralbl. f. Bakt.*, 1938, **142**, 269.

* We are indebted to Dr. S. Kasahara of the Kitasato Institute, Tokyo, Japan, for several strains of Japanese virus. The Nagayama strain has been used in these experiments.

Microscopically the mesoderm of the membrane is edematous following the inoculation of either virus. There is a moderate proliferation of the fixed mesodermal cells and some infiltration of wandering cells. The latter frequently occur in foci about vessels or just beneath the ectodermal layer of cells. No specific cellular inclusions have been seen in membranes inoculated with either virus.

When the virus of St. Louis encephalitis is used as the inoculum the majority of the chicks, at least in early passages, remain alive until approximately the time of hatching. When the Japanese encephalitis virus is used most of the chicks remain alive at least 5 to 7 days. Little change has been observed in the brains of chicks following inoculation of the chorioallantoic membrane with either virus. In both instances a few foci of mononuclear wandering cells have been observed in the meninges. Chick brains from the first, second and sixth egg passages of the Japanese encephalitis virus have shown no other changes. Following the inoculation of the St. Louis encephalitis virus, in addition to the slight meningeal reaction, small foci of glial proliferation have been observed, but in only a few instances.

Titration of the virus content of the chorioallantoic membrane and of the brain of chick embryos inoculated with St. Louis encephalitis virus have been carried out on a number of occasions. Even after many passages, the degree of multiplication of the virus in the egg as determined by mouse inoculations of serial dilutions, has varied little. The chorioallantoic membrane is uniformly infectious for mice in broth dilutions of 10^{-2} , irregularly so in dilutions of 10^{-3} . The chick brain is infectious for mice uniformly in dilutions of 10^{-3} and occasionally in dilutions of 10^{-4} .

The chorioallantoic membranes and the brains from the 5th egg passage of the Japanese encephalitis virus have been tested for their virus content by mouse inoculation with results quite comparable to those obtained following inoculation with the St. Louis encephalitis virus. Mice receiving the 10^{-1} and 10^{-2} broth dilutions of the membranes died in 5 to 8 days after inoculation. One of 3 mice receiving the 10^{-3} dilution of the membrane died. Mice receiving the 10^{-1} , 10^{-2} , and 10^{-3} dilutions of chick brain died in 5 to 8 days, while 2 of 3 mice receiving the 10^{-4} dilution survived.

Following inoculation of the chorioallantoic membrane with the St. Louis encephalitis virus, the virus could also be demonstrated in other organs including the lungs, kidneys, spleen, and liver. Following the inoculation of the chorioallantoic membrane with the Japanese virus, only the liver of chick embryos has been tested in addition to the

membrane and brain. A 10% suspension of this organ in broth was infectious for mice.

Conclusion. The Japanese encephalitis virus and the St. Louis encephalitis virus produce the same type of changes in the chorio-allantoic membrane and in the brain of the chick embryo. The two viruses multiply in the egg to approximately the same titer as demonstrated by mouse inoculation. Whether these observations imply more than a similarity of action of the two viruses is not clear from data available from this study.

10661 P

Contractions of Frog's Gall Bladder and Its Possible Use as an Assay Method.

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Contraction of the frog's gall bladder can be readily demonstrated by the intracardiac injection of crude secretin. The frog's gall bladder is usually bluish green, moderately full of bile, pear-shaped, flabby, and is easily observed when the abdomen is opened. After the intracardiac injection of a dilute secretin preparation, there is a latent period of 15 to 100 seconds; the gall bladder then changes to a rounded spherical form, and the organ develops a slight or marked opalescence; the surface may also show a slight or marked puckering; blood vessels over the surface may become tortuous; apparent volume changes may at times be noted. After 2-10 minutes, the surface again becomes smooth, opalescence disappears, and the bladder becomes flabby.

The ease with which the above could be duplicated suggested its use as an assay method for substances contracting the gall bladder.

A number of preparations have been compared using the following procedures as standard. Active dark colored male frogs weighing 25-35 g were selected; the cerebra were crushed, the cords pithed, and the animals pinned out on frog boards; the feet of the frogs were elevated 1-2 inches above the heads and the gall bladders were exposed; the blood flow to the gall bladder was directly observed microscopically and only preparations were used which showed a good circulation.

Secretin powder S I, (Ivy) which is relatively stable, was used as a standard.

A unit has been arbitrarily defined as the amount of gall bladder contracting material present in 0.2 cc of solution which, when injected intracardially into 30 g frogs, brings about contraction in 50% of 30 experiments.

The activities of 3 crude secretin preparations from dog duodena and a powdered cholecystokinin, prepared according to Ivy's pH 1802 method, have been compared to the standard S I powder. The powders were used in strength of 0.030, 0.025, 0.020, and 0.015% for the S I and 0.1 and 0.2% for the cholecystokinin. The crude secretin preparations were used in dilutions of 1:30, 1:25, 1:20 and 1:15. It was found that 0.2 cc of a 0.020% S I solution gave contraction in 47% of 32 experiments. Cholecystokinin powder in 0.1% solution gave contraction in 19% of 16 experiments while in a 0.2% solution, it gave a gall bladder contraction in 80% of 20 experiments. The crude secretin preparations A, A₁, and B in a dilution of 1:25 gave gall bladder contractions respectively in 50% of 8 experiments, 56% of 32 experiments, and 47% of 30 experiments. Converting these results into terms of units, 1 mg of S I would be the equivalent of 25 units. 1 mg cholecystokinin would be the approximate equivalent of 3 units. Undiluted secretin preparations would contain the equivalent of approximately 125 units per cc or the equivalent of 5 mg of S I per cc.

10662 P

Immunization of Mice by Intranasal Instillation of Nasopharyngeal Washings from Cases of St. Louis Encephalitis.*

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The 1937 outbreak of encephalitis in St. Louis afforded an excellent opportunity for further study of the disease. Despite the fact that the virus of encephalitis has never been demonstrated in nasal secretions procured from patients during the acute phase of the

* This work was made possible by a grant from Special Fund for the Study of Encephalitis.

disease,^{1, 2} it is held by some workers that the mode of spread is by way of the upper part of the respiratory tract. It is well known that the disease is easily produced in mice by the instillation of mouse-brain virus directly into the nares. Furthermore, Armstrong³ has reported that 30 to 60% of mice which had withstood an intranasal instillation of the virus may have become immune and would survive a subsequent intracerebral injection of an amount sufficient to kill normal controls.

In the present experiments nasopharyngeal washings from patients with the disease were given intranasally to mice on 2 successive days in order to attempt to demonstrate the presence of active virus. This route was used since material containing bacteria can be used as the inoculum, thus avoiding possible loss of virus by filtration or other means of removing the contaminating bacteria. Since none of the mice receiving intranasal instillation of nasopharyngeal washings developed recognizable symptoms of the disease, we attempted to determine whether any immunity was conferred by these instillations.

About 3 weeks after the last instillation, the mice were given an intracerebral inoculation of an amount of virus which was sufficient to kill all (20) control mice. Of the 40 test mice, 14 (35%) survived the injection. This observation encouraged us to make further trials. Accordingly, washings from 15 patients during the acute stage of the disease were instilled into the nares of a series of mice. However, in these experiments 7 to 8 instillations were made. None of these animals developed clinical signs of the disease, but a similar test for immunity showed an even higher incidence of survivors. Of the 164 test mice, 81 (49%) survived the subsequent intracerebral injection, while all of the 54 control mice died.

These experiments suggest that virus is present in the secretions of the upper respiratory tract during the clinical disease and the mode of transmission may be by means of such discharges.

Since the above findings were obtained, no further clinical cases have been available for study so that there has been no opportunity to repeat these experiments on a larger scale. We are reporting them at this time because of the possibility that cases may occur again this summer, at which time other workers might attempt the detection of the virus in the nasopharyngeal washings of patients and particularly of contacts.

¹ Muckenfuss, R. S., Armstrong, C., and McCordock, H. A., *Public Health Report*, 1933, **48**, 341.

² McCordock, H. A., Smith, M. G., and Moore, E., *Proc. Soc. Exp. Biol. and Med.*, 1937, **37**, 288.

³ Armstrong, C., *Public Health Report*, 1934, **49**, 959.

We wish to express our appreciation to Doctors T. R. Young, N. J. Bublis, W. O. Gorla, and M. Bartnick of the St. Louis Isolation, Missouri Baptist, St. Mary's and St. Anthony's Hospitals, respectively, for placing at our disposal the clinical material for this investigation.

10663 P

Attempts to Infect Guinea Pigs with the Virus of St. Louis Encephalitis.*

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It is known that passage of influenzal virus directly from human patients to mice is extremely difficult,¹ but that passage to ferrets and then to mice is readily accomplished. It is also probably true that passage of smallpox virus directly from patients to calves or rabbits is difficult, while previous passage through monkeys converts the virus to vaccinia which is then more readily infectious for calves and rabbits.^{2, 3, 4} Reasoning by analogy, it was suspected that susceptibility to St. Louis encephalitis virus might be transmitted to a wider variety of animals if brain-tissue of suitable animal species were used as inoculum. In spite of the fact that previous attempts to infect guinea pigs with human brain material^{5, 6} or mouse-brain virus^{7, 8} have been unsuccessful, we have considered this worthy of another trial with the view of using guinea pig brain virus for the inoculation of other animals.

In the first series of experiments, begun in the fall of 1937, mouse-brain virus suspended in Locke's solution was introduced intra-

* This work was made possible by a grant from Special Fund for the Study of Encephalitis.

¹ Francis, F., Jr., and Magill, F. P., *PROC. SOC. EXP. BIOL. AND MED.*, 1937, **36**, 132.

² Gordon, J. H., *London Med. Res. Council, Sp. Reg. Ser.*, No. 98, 1925.

³ McKinnon, N. E., and Defries, R. D., *Am. J. Hyg.*, 1928, **8**, 93.

⁴ Leake, J. P., and Force, *Pub. Health Rep.*, 1921, **36**, 1437.

⁵ *Public Health Bulletin*, No. 214, 1935, p. 28.

⁶ McCordock, H. A., Smith, M. G., and Moore, E., *PROC. SOC. EXP. BIOL. AND MED.*, 1937, **37**, 288.

⁷ Brodie, M., *PROC. SOC. EXP. BIOL. AND MED.*, 1934, **31**, 1229.

⁸ Webster, L. T., and Fite, G. L., *J. Exp. Med.*, 1935, **61**, 411.

cranially into 2 guinea pigs. After incubationary periods of 14 and 17 days, both animals were found dead. The brains of these guinea pigs were in turn inoculated into animals, and in a similar way the virus was carried through 6 passages as shown in the accompanying diagram. As may be seen, some were found dead, while others were sacrificed when showing convulsions. On several occasions the brains from these animals were passed back to mice, the latter ani-

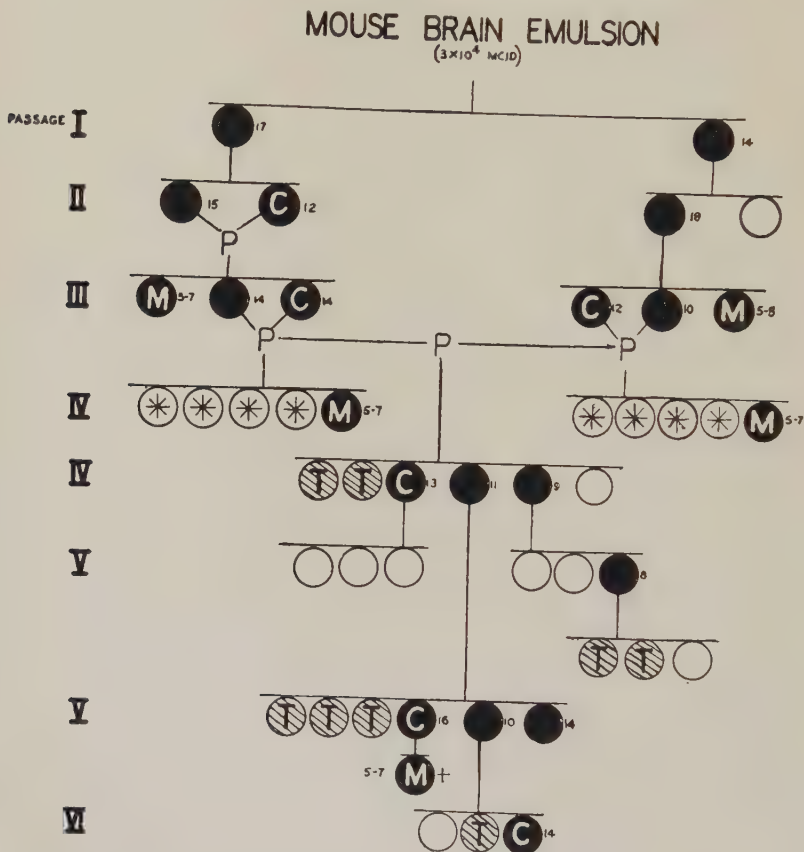


FIG. 1.

Solid black—Guinea pig found dead.

Black with letter "C"—Guinea pig in convulsions.

Black with letter "M"—Inoculated into 6 mice mixed with normal serum and into 3 mice mixed with human convalescent serum. Result: 6 inoculated with normal serum mixtures died with signs of encephalitis; the other 3 survived.

Black with M+—3 mice injected; all died with signs of encephalitis.

Shaded with letter "T"—Guinea pig died of trauma.

Solid white—Guinea pig survived.

P—Guinea pig brain material pooled.

*—Guinea pigs came from the dealer the same day and all survived.

Figures indicate length of incubation period in days.

mals developing typical signs of encephalitis which could be prevented by neutralization with convalescent sera. It may be noted particularly that on one occasion all of the guinea pigs purchased on a certain day survived while the same material injected into others at a later time produced encephalitis in 3 out of 4 animals.

In another series of experiments, in the spring of 1938, the virus was successfully carried through 5 consecutive passages in guinea pigs with essentially similar results. In addition, sections of brains of several guinea pigs in this series, including one from the fifth passage, showed histopathological evidence of encephalitis. However, when we attempted to carry the experiments further in the fall of 1938, we were unable to infect guinea pigs either with the brain of guinea pigs (preserved in glycerin and from the previous experiment), or with freshly passaged brains of mice. Several variations in technic were employed without success; these included the use of more concentrated brain-suspensions, uncentrifuged suspensions, and normal guinea pig serum as diluent.

In a personal communication, Doctor Max Theiler reported a similar experience with yellow-fever virus and found that the susceptibility to this virus depends on the genetic strain of guinea pigs used. Likewise, Sonto and his associates⁹ have found a difference in susceptibility of different strains of guinea pigs to tetanus toxin. Since such a factor may have operated in our experiments, we are planning further trials with different strains of guinea pigs.

We wish to express our indebtedness to the late Doctor H. A. McCordock for pathological examination of the tissue of the guinea pigs.

10664 P

Susceptibility of Wild Mice to the Virus of St. Louis Encephalitis.*

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For the last 2 years we have been concerned with the question of the possibility of a reservoir in animals of the virus of St. Louis

⁹ Sonto *et al.*, *Revue d'Immunologie*, 1939, **5**, 54.

* This work was made possible by a grant from Special Fund for the Study of Encephalitis.

encephalitis. Since this has been an interepidemic period, our method of study has been to determine experimentally the susceptibility of various species. Among others, wild gray house-mice (*Mus musculus*) have been tried and found susceptible.

Up to the present, virus has been carried (by intracerebral inoculation) through 10 passages in wild mice and has retained its infective titer as tested on Swiss mice. Furthermore, a test with rabbit antiserum showed specific protection against passage virus. In addition to the intracerebral route we have also succeeded in transmitting the infection to wild mice by the intranasal route.

In another series of experiments we have found it possible to transmit infection to Swiss mice by feeding infected material but in a single experiment so far performed have failed to infect a wild mouse by this route. The investigation is still in progress and this fragmentary report is made at this time because of the possibility of human cases occurring again this summer, at which time it would be desirable to look for virus in wild mice trapped in the households where cases have occurred.

10665 P

Infection of Mice by Feeding of Tissues Containing the Virus of St. Louis Encephalitis.*

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In attempts to determine whether the injection of young mice would serve as a method for the detection of smaller amounts of virus, newly born, unweaned Swiss mice have been inoculated intracerebrally or intranasally with tenfold dilutions of suspensions of infected adult mouse brains. These mice have been found susceptible and preliminary experiments suggest the possibility that newly born mice will succumb to one-tenth the dose necessary to kill grown mice by the respective routes.

In the course of these experiments it has been noted frequently that the mothers had devoured their dead or moribund offspring. In 3 of such cases the mothers were found dead after intervals of 5 to 7

* This work was made possible by a grant from Special Fund for the Study of Encephalitis.

days. This suggested the possibility of the mothers having contracted the infection by feeding and accordingly the brain of the last one was tested by passage to other mice and found to contain virus. In addition, grown mice of both sexes were allowed to feed on moribund or dead, newly born mice previously infected with virus by the intracerebral route and in 2 of 7 instances the mice died or were sick 7 days after feeding. From these, virus was obtained from the brain by passage to grown mice. A few experiments performed so far, in which mice have been allowed to eat infected adult mouse brains, have all failed to result in clinical infection. This is in agreement with the findings of Brodie.¹

The importance of the observation that adult mice can be infected by eating the bodies of infected, newly born mice, is that this mode of entry may have epidemiological significance. It is the only mode of entry so far described in which this infection has been transmitted from mouse to mouse by some natural means and not by inoculation. It is particularly important in conjunction with the fact that the wild mouse is susceptible to the virus.

10666 P

Relation of Methionine, Cystine and Choline to Renal Lesions Occurring on Low Choline Diets.

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In a previous report¹ it was demonstrated that a severe pathological state characterized by hemorrhagic degeneration of the kidneys occurs within 10 days in young rats maintained on a low choline diet. This deficiency was prevented by choline. It was suggested that proteins relatively high in methionine and low in cystine possessed a choline-sparing action since the deficiency was produced more readily on diets containing fibrin than on those containing casein. Subsequent work has confirmed the earlier suggestion that choline might prevent the renal lesion resulting from the addition of cystine to a purified diet containing casein. Furthermore, it has been found

¹ Brodie, M., *PROC. SOC. EXP. BIOL. AND MED.*, 1935, **32**, 1647.

¹ Griffith, W. H., and Wade, N. J., in press.

that the addition of methionine to a diet containing fibrin completely protected the rats.

Normal kidneys were found in 40 g rats fed the following diet for 10 days: casein, 15; salt mixture, 4; calcium carbonate, 1; codliver oil, 5; lard, 35; agar, 2; sucrose, 32, and yeast, 6. Hemorrhagic kidneys invariably occurred if 0.3% cystine was added but not if 0.1% choline was added in addition to the cystine.

Hemorrhagic kidneys resulted if the protein of the above basal ration consisted of fibrin, 4; casein, 8, and dried egg white, 3. The addition of 0.04% choline or of 1% dl-methionine completely protected the rats.

The ratio of the 2 amino acids, methionine and cystine, is not the only factor which determines the choline requirement. This became evident from the fact that hemorrhagic lesions were produced on the 15% casein diet by decreasing the level of choline in the diet through substitution of vitamin concentrates for the codliver oil and yeast and through lowering the fat content to 10%. Furthermore, the effect of a fibrin diet in producing the renal lesions was no longer evident if the fibrin was decreased from 15 to 5%. It is suggested that the absolute amount of either methionine or cystine, as well as the ratio of the 2, plays an important rôle in the interrelationship of these 2 amino acids and choline.

10667

Nitrogen-Containing Carcinogenic Compounds.

LIONEL JOSEPH. (Introduced by E. V. Cowdry.)

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Of all the heterocyclic nitrogen compounds studied, up to the present, none has been related to the carcinogenic hydrocarbons, such as methylcholanthrene, to the extent of containing an anthracene or phenanthrene nucleus. In view of the pronounced carcinogenic action of these hydrocarbons and in view of the fact that certain nitrogen compounds *not* related to these hydrocarbons have been found to be carcinogenic it seemed advisable to study the carcinogenic effect of heterocyclic nitrogen compounds related to these hydrocarbons to the extent of containing an anthracene or phenanthrene nucleus. Further, since certain indole derivatives which are not related to the car-

cinogenic hydrocarbons have been shown to be carcinogenic it seemed of interest to prepare an indole derivative which would be related to a carcinogenic hydrocarbon and to test its carcinogenic activity. For this purpose 2,9(N)-indoloanthrone was prepared. This compound is readily reduced to the anthranol which is then analogous to a hydroxybenzpyrene. However the reduced form could not be obtained pure due to the rapid oxidation by atmospheric oxygen. Therefore the oxidized form of the compound was used in the hope that this might be reduced by the body tissues.

The other heterocyclic compounds studied were 1,2(N)-pyridinoanthracene, 1,2(N)-pyridinoanthracene methiodide, 3(N), 4-pyridinophenanthrene, and 3(N), 4-pyridinophenanthrene methiodide.

In addition it was decided to test 3-aminophenanthrene. This was done because of the observation made by Shear¹ that 2-aminoanthracene was capable of producing liver tumors and it was felt that 3-aminophenanthrene should be more effective than the corresponding anthracene derivative because, in general, phenanthrene derivatives are more efficient than anthracene derivatives.

2,9(N)-indoloanthrone was prepared by the method of Scholl.² Naphthalene was condensed with phthalic anhydride in the presence of aluminum chloride. Ring closure was effected by means of concentrated sulphuric acid and the resulting benzanthraquinone nitrated with a mixture of acetic anhydride and fuming nitric acid. The 2 nitro compounds were separated by fractional crystallization from chloroform and recrystallized from benzene. The 2-nitrobenzanthraquinone was treated with phenylhydrazine to give the desired compound.

Pyridinoanthracene was prepared by first reducing beta-aminoanthraquinone to beta-aminoanthracene according to the method of Braun and Bayer,³ then treating the product with glycerin and concentrated sulphuric acid to yield the desired product, m.p. 169°C. The methiodide of this material was obtained by warming equivalent amounts of pyridinoanthracene and methyl iodide in 5 volumes of absolute alcohol for several minutes and recrystallizing the product from 95% alcohol; m.p. 221°C dec.

Pyridinophenanthrene was prepared according to the method of Mosettig and Krueger⁴ by treating 3-aminophenanthrene with glycerine and sulphuric acid. The methiodide was prepared in the

¹ Shear, M. J., *Am. J. Cancer*, 1937, **29**, 269; *J. Biol. Chem.*, 1938, **108**, 123.

² Scholl, R., *Ber. d. Deut. Chem. Ges.*, 1911, **44**, 2370.

³ Braun, J., and Bayer, O., *Ann. d. Chem.*, 1929, **116**, 472.

⁴ Mosettig, E., and Krueger, J. W., *J. Am. Chem. Soc.*, 1936, **58**, 1311.

same manner as pyridinoanthracene methiodide; m.p. 239-240°C dec. The 3-aminophenanthrene used in this preparation was prepared from phenanthrene by the method of Bachmann and Boatner.⁵

A 2% solution of each of these compounds in benzene was painted twice weekly on the neck of 100 mice over a period of approximately 5 months. In addition to this .02 g of each of the compounds was suspended in 0.5 cc of paraffin and this dose was planted subcutaneously in 20 mice. These animals were observed for approximately 7 months. Neither in the instance of the skin paintings nor in those of the subcutaneous implantations did carcinoma occur.

Though certain polycyclic hydrocarbons of the benzanthracene type are carcinogenic, and despite the fact that certain nitrogen-containing compounds have also been found to be carcinogenic, these compounds, prepared and tested containing nitrogen and chemically analogous to benzanthracene, are probably non-carcinogenic. This observation serves to render our concept of carcinogenic substances more precise.

10668

Aldehydic Resorption in Mice.

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Kudrjashov and Agatov¹ reported that they were able to induce temporary sterilization, or failure of implantation, in female rats and rabbits by means of fractions obtained from rancid fat and oleic acid. The rancid fat did not act upon the sexual system of the rat, but caused the death of the embryo at or soon after implantation, the placental sign occurring on the 9-10th day (normal 13th day). The authors believe that the active substance may consist of aldehydes and ketones.

Strong's² very interesting observations on the liquefaction and regression of spontaneous mammary tumors by means of heptaldehyde have aroused considerable interest, and any light on the mechanism of heptaldehyde on tumors should be welcome at this time. He³

⁵ Bachmann, W. E., and Boatner, C. H., *J. Am. Chem. Soc.*, 1936, **58**, 2097.

¹ Kudrjashov, B. A., and Agatov, P. A., *Ginekologia i Akusherstvo*, 1935, **6**, 1.

² Strong, L. C., *Am. J. Cancer*, 1939, **35**, 401.

³ Strong, L. C., *Science*, 1938, **88**, 11.

has also noted that this aldehyde can cause resorption of the embryos in mice, but no details have been given. Kudrjashov and Agatov's observations may be explained by the fact that heptaldehyde is one of the constituents of rancid fat.⁴

Experiments were undertaken to study the resorptive effectiveness of various substances, especially heptaldehyde. Old Buffalo, New Buffalo,* C57 (black) and market mice were used. Purina Dog Chow was fed *ad libitum*. Six to 8 females were penned with one male. Vaginal smears were made daily, and the animals weighed every 5 days.

In the first experiment the substance to be tested was fed orally with a medicine dropper at various days after a positive mating. Of the following substances fed (40-50 mg daily) from the first to the fourth day after insemination to a resorption or successful pregnancy, unsaponifiable fraction of rancid lard, propionaldehyde, n-butraldehyde, n-valeraldehyde, heptaldehyde, pelargonic aldehyde, and benzaldehyde, only heptaldehyde gave marked results (Table I). Benzaldehyde, n-butraldehyde and pelargonic aldehyde induced an occasional resorption. When a resorption did follow the feeding of an aldehyde, no more was fed until the mouse had had a successful gestation.

The administration of heptaldehyde causes an earlier appearance of the placental sign (extremes 5-12 days) when a resorption follows than when a pregnancy ensues. The amount of blood was somewhat variable, from a small amount of light colored blood to a more copious exudate of dark stringy masses. The placental sign usually lasted from one to 4 days, sometimes longer. The aldehyde was fed

TABLE I.
Resorptive Effectiveness of Heptaldehyde Fed Orally.

Fed at days after insemination	Resorptions			Pregnancies		
	Avg			Avg		
	Placental sign, days	No. of days aldehyde fed	No. of mice	Placental sign, days	No. of days aldehyde fed	No. of mice
0	7	8	3	11	16	1
1	8.1	8.3	28	9.3	9.5	9
2	7.5	7	2	10	14	4
3	7	3	1	10	12	2
4	7	3	1	10	16.4	8
5	5	12	1	9.5	14.5	2

⁴ Powick, W. C., *J. Agri. Res.*, 1923, **26**, 323.

* Generously supplied by The New York State Institute for the Study of Malignant Diseases, Buffalo, New York, Burton T. Simpson, Director.

until the estrous cycle returned, or until it could be seen that the feeding was going to be ineffective (10-16th day). The placental sign appears in normals on the 9th to the 13th day. It was impossible to induce resorptions when the administration of the aldehyde was started after the 9th to 13th days after insemination. Resorptions did not occur in all the mice presumably due to individual variations in the amount required to destroy fertility. Oral feeding rarely caused a loss in weight, but heptaldehyde caused a loss of hair around the mouth and face of the mice.

Since heptaldehyde was incapable of causing resorptions, either in all the mice or after the 9th to the 13th day after insemination, when fed orally, it was assumed that some of the aldehyde had been destroyed prior to absorption. To test this assumption the ethyl esters of lard were made according to the method of Olcott and Mattill⁵ and a solution of the aldehyde in the esters was prepared so that .2 cc esters contained .02 cc heptaldehyde. With this solution injected intraperitoneally the mice could tolerate .02 cc of the aldehyde daily throughout the entire gestation period. Similar injection of the aldehyde alone proved highly toxic and subcutaneous injections resulted in necrosis. Lethal doses of the aldehyde, in the ethyl esters of lard, ranged from .08 to .09 cc. Usually .02 to .06 cc of the aldehyde was injected daily at different days after a positive mating (Table II).

The results are particularly interesting, because resorption could be induced as late as the 13th day after insemination with as little as .10 cc heptaldehyde. Resorptions later than the 13th day have been obtained, but death usually followed. Resorptions were easily induced from the first to the fourth day after insemination by .08 cc of the aldehyde. The entire process was usually completed in 3 to 7 days. If oral feeding did not induce resorptions by the 11-13 days, they could be induced by several injections (.04 to .12 cc of the aldehyde). These results would seem to indicate that little of the aldehyde, administered orally, reaches the blood stream. In agreement with this is the fact that the oral feeding of heptaldehyde had no effect on the growth of the Marsh Carcinoma. This may be due to poor absorption or destruction of the aldehyde before it reaches the tumor.

Resorptions were easily caused by the intraperitoneal injections of heptaldehyde in the ethyl esters of lard if the mice had not received the aldehyde orally or intraperitoneally previously. Of 17 young females (New Buffalo and C 57 [black]), which had received no

⁵ Olcott, H. S., and Mattill, H. A., *J. Am. Chem. Soc.*, 1936, **58**, 2204.

TABLE II.
Resorptive Effectiveness of Heptaldehyde Dissolved in the Ethyl Esters of Lard.

Injections begun at days after insemination	Resorptions Placental sign, days			No. of mice	Pregnancies Placental sign, days			cc aldehyde injected		No. of mice
	Avg	Extremes			Avg	Extremes		Avg	Extremes	
1	8.2	6-13		7						0
2	5.5	5-6		2						0
3	7			1						0
4	5.8	4-7		6						0
8	8			1	10			.20		1
9	9			1						0
10	11.5	10-13		2	10			.14		1
11	11	11		4	11	11		.16	.12-.20	4
12	12	12		2	13			.20		1
13	13	13		5	13			.12		1

aldehyde previously, 15 resorbed their embryos; while of 25 females, which had received the aldehyde orally or intraperitoneally (or both), 11 had resorptions, when the intraperitoneal method was used. Many mice failed to respond to a second oral administration of heptaldehyde, although pregnancy could be terminated in some of these cases by intraperitoneal injections. Apparently the mice are capable of building up a resistance to heptaldehyde. Even the ethyl esters of lard, which had become rancid, could stop pregnancy from the 4th to the 13th day after insemination with a dose of .3 to .6 cc injected intraperitoneally. Intraperitoneal injections of the other substances previously mentioned have not been tried.

Of 464 positive matings throughout these experiments, 86 (18.5%) were not followed by implantation. Heptaldehyde brought about a large number of these failures, and the other aldehydes were also capable of similar action. There were 264 successful gestations (with and without aldehydic administration). The sexual system of the mouse is apparently not impaired, because fertility returns after cessation of the aldehydic treatment. Whether the resorption of embryos and the liquefaction and regression of spontaneous tumors by heptaldehyde (Strong) are expressions of a similar phenomena—destruction of vitamin E—remains to be determined. The work of Kudrjashov⁶ indicates that the fractions obtained from rancid fat act directly on the embryo and do not destroy vitamin E.

Conclusions. Heptaldehyde is capable of inducing resorption of mouse embryos when it is administered orally from the first to the fifth day after insemination, but it is most effective on the first and second days after insemination. Dissolved in the ethyl esters of lard, it can cause destruction of fertility as late as the thirteenth day after insemination. The oral feeding of heptaldehyde had no appreciable effect on the growth of the Marsh Carcinoma, but these experiments indicate that further work is necessary to determine the best conditions whereby heptaldehyde (or other aldehydes) can have greater access to tumors before its therapeutic value can be established.

⁶ Kudrjashov, B. A., *Arch. f. exper. Path. und Pharmacol.*, 1932, **169**, 275.

10669

Physical and Chemical Properties of Rat Leprosy Bacilli.*

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It is known that percentages of serum Ca and P are not noticeably altered in human leprosy except in unusual cases or during the lepra reaction. In order to discover the changes, if any, in the lesions themselves a histospectrographic investigation was made of skin lesions obtained from Dr. O. E. Denney at the U. S. Marine Hospital, Carville, La. It was found¹ that the P/Ca ratios in the 5 cases studied were probably 3 times those in normal skins from the same age group. A fair correlation was obtained for the P/Ca ratios with known duration of the disease and volume of leprous cells in the tissues analyzed spectroscopically. The change from the normal may be conditioned by increase in P, decrease in Ca, but probably by both. Perhaps an increase in P may have been occasioned by the tremendous number of bacilli in the lesions. No notable deviations from normal were noted in the Na/Ca, Mg/CA and Fe/Ca ratios.

In the hope of relating these observations on mineral constituents more definitely to cells and groups of cells, the technic of microincineration² was applied to sections of human leprous nodules also secured from Carville. We found that the lepra cells showed a finely divided white ash, tending in some places to be slightly bluish and something like that observed in cancer cells.³ However, we were not successful in differentiating the ash resulting from the bacilli on the one hand and from the cells containing them on the other.

Consequently we shifted the attack to sections of rat leprosy nodules with which it was obviously more easy to experiment in our laboratory. The strain of organisms was received from Dr. E. L. Walker, 12/11/34. The tissues were either fixed in 10% formalin in absolute alcohol or were frozen in liquid air and dehydrated while still frozen in a cryostat. Alternating sections were stained with Ziehl-Neelson-hematoxylin and were incinerated. Nevertheless, it

* Aided by a grant from the U. S. Public Health Service.

¹ Cowdry, E. V., Heimbürger, L. F., and Williams, P. S., *Am. J. Path.*, 1936, **12**, 13.

² Scott, Gordon H., *McClung's Microscopical Technique*, 2d Edition, 1937, New York, Hoeber, pp. 643-666.

³ Scott, G. H., and Horning, E. S., *Am. J. Path.*, 1932, **8**, 329.

was often difficult to identify in the incinerated section the same cell, or group of cells, which had been stained for bacilli in the next section because there are so few landmarks in the nodules. What we did was to concentrate our attention on relatively large intracellular aggregates of bacilli known as rosettes.⁴ In no instance could we locate any mass of ash corresponding to a rosette. We conclude, therefore, that the finely divided white ash residue in lepra cells is mainly an expression of the mineral constituents of the cytoplasmic ground substance and not of the intracytoplasmic bacilli.

Influenced by reports on differences in fluorescence of different tissues and of different kinds of bacilli (summarized by Radley and Grant⁵) we assembled a fluorescence-microscope with which to ascertain whether any significant alterations occur in leprous lesions or in the bacilli. An old Zeiss microscope was fitted with a quartz condenser. Light from a General Electric high-pressure mercury lamp (type H-4 with outer envelop removed) was directed into the condenser by a quartz 90° prism. A Wood's filter, 5 mm thick, placed between the arc and the prism, eliminated all visible light but the violet. The latter was removed by placing a Bausch and Lomb fluorescent filter over the ocular of the microscope. It is important to note that, when a Wood's filter 3 mm thick is employed, as by some investigators, much more violet and blue light will be admitted from the mercury arc so that some confusion will result in the proper description of the color of fluorescence. Since the filter over the ocular also depresses the blue, it enhances the other spectral colors. The conditions must be very carefully specified in order for the observations to be properly interpreted and compared with those made by other investigators.

Rat leprosy nodules were fixed in 10% formalin, dehydrated, cleared, and embedded in paraffin in the usual way. Serial sections were usually cut 4 microns thick. In special cases the thickness was 3 microns. They must be thin for otherwise the fluorescence is so intense as to hide structural details. For the sake of orientation in the lesion, sections alternating with those for fluorescent study, were stained and mounted in the usual way. After the others, intended for fluorescence, had been mounted on quartz or Corex-D slides, the paraffin dissolved with xylol and the xylol allowed to evaporate they were ready for examination.

⁴ Cowdry, E. V., and Ravold, Amand, *Puerto Rico J. Pub. Health and Trop. Med.*, 1938, **2**.

⁵ Radley, J. A., and Grant, G., *Fluorescence Analysis in Ultraviolet Light*, London, Chapman & Hall, Ltd., 1935, 326 pp.

The color of fluorescence of the nodule was found to be grayish white with a definite greenish cast. The light emitted was not as strong as that from the liver, myocardium, kidney, pancreas, and other normal tissues used for comparison, and consequently it was a little less white. These other tissues all gave a grayish-white fluorescent color with a greenish cast, but the green was not nearly as pronounced as in the case of the leprous nodule. Although the leprous tissue had many bacilli, none of them could be distinguished as such. It is likely, however, that the definite greenish color of the fluorescence was partly due to the great abundance of the bacilli. Despite this characteristic appearance under ultraviolet illumination, leprous tissues in our sections could not be analyzed by the fluorescent method with any accuracy.

Evidently, in order to extend the observations on fluorescence as well as on mineral constituents, it was necessary for us to separate the bacilli from the lesions. This was done by employing a technic devised by Ravold according to which relatively large masses of bacilli-laden cells are dissected away from neighboring uninvolved tissue and from necrotic tissue when present in the centers of the nodules. They are placed in a Wueller press without addition of any fluid. On exertion of pressure many of the cells are ruptured and the tissue fluid, together with cytoplasm, nucleoplasm and some entire cells, passes through minute holes in the press and is collected, leaving most of the fibrous elements behind. Then a little saline solution is added and the material is ground up in sand and made up to a volume of about 50 cc. The sand is allowed to sediment out at the bottom of a centrifuge tube. The supernatant fluid is then centrifuged at low speed (300 r.p.m.). This throws all the rest of the debris to the bottom while the bacilli remain in suspension. The supernatant fluid, containing the bacilli, is again decanted and centrifuged at high speed (3500 r.p.m.) in an angle centrifuge for 1 hour. The supernatant fluid is discarded and the pasty material at the bottom of the tube, made up of bacilli, is diluted and washed by repeated centrifugation in some experiments with saline solution and in others with distilled water.

Beginning with a large nodule or with several small ones it is a simple matter to collect in 4 or 5 hours billions of bacilli. The pasty bacterial mass can be desiccated and weighed in grams. For our experiments we used only the wet bacilli, when viewed *en masse* they appear dense white with a faint shade of gray. They are not yellow or even yellowish. Examination of a thick smear, made after washing in saline, shows myriads of bacilli without any trace of cellular

material. The bacilli retain to a remarkable degree their characteristic morphology, as seen in sections and in smears of fresh tissue, and their acid-fast properties are not interfered with. After washing in distilled water until the supernatant fluid gave no precipitate when added to an aqueous solution of silver nitrate, the bacilli do not fuse together but still remain discrete bodies though their shape is different.

Despite the most stringent aseptic precautions, an organism other than *Mycobacterium lepræ muris* was detected both microscopically and culturally in some samples which were then discarded. Thus far we have not determined whether this organism occasionally occurs in the leprous nodules or whether it is a contaminant from the air.

Thick smears of the bacilli, washed in distilled water, gave little or no mineral residue after microincineration which lends support to the conclusion that the residue of sections of cells crowded with bacilli is mainly due to cellular components other than the bacilli. However, by the much more sensitive method of spectrographic analysis, some minerals were detected in the bacilli. We have not completed these analyses as yet, and do not know all that they will tell us, but in the spectrograms of washed bacilli, as compared with those of controls of fluid, obtained when normal tissues were treated in the same way as the lesions, it was found (1) that there is a significantly greater P/Ca ratio due to a considerable increase in P and to a lesser decrease in Ca. (2) That the bacilli contain traces of sodium and magnesium in about the same amounts as in the controls. (3) That traces of K are very slight in both the bacilli and in the controls, if anything less in the former.

In ultraviolet light the masses of distilled-water-washed bacilli on the special slides fluoresced faintly white definitely tinged with green. We believe, therefore, that the greenish fluorescence of cells charged with bacilli seen in sections is to be attributed chiefly to the bacilli. Unfortunately this observation, though interesting if other bacilli could be examined in precisely the same way, does not permit any deductions as to the chemical composition of the organisms. While every fluorescent substance has a characteristic color under ultraviolet light, the visual appearance of a great many compounds is so similar that accurate conclusions, regarding the constitution of the fluorescent material cannot be drawn. Spectrographic examination of the fluorescent emission is the only reliable method of fluorescent analysis and for this we are not equipped.

In conclusion, the available evidence indicates that the mineral ash of leprous nodules is mostly due to the cells of the host and that the

green shade of fluorescence is due mainly, but not entirely, to the contained bacilli. It is possible that the method devised for separating bacilli from the lesions and for collecting them in large numbers, which we have found useful in rats, may be helpful when extended to leprous lesions of humans.

10670 P

Influence of Anoxia on Glycogenolytic Action of Adrenalin.*

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In the course of a study of the interaction of hypoglycemia and anoxia in the rabbit¹ it was found that a short period of anoxia (7% oxygen for 15 minutes) enhances the return of the blood sugar to the control level, whereas the inhalation of 7% oxygen for 2 hours greatly aggravates the hypoglycemia. In spite of the fact that in the latter group the blood sugar averaged less than 30 mg % and was maintained at this level for 2 hours, no convulsions occurred, thus confirming McQuarrie and Ziegler's² experiments. The question was studied whether the differential reaction of the blood sugar to anoxia in the 2 groups of experiments is related to the effect of adrenalin on the liver. If this were the case a greater hyperglycemic effect of adrenalin would be expected after a short period of anoxia than is observed under control conditions. Furthermore, prolonged periods of anoxia should lead to a diminished glycogenolytic response of the liver.

Fifty-six experiments were carried out on rabbits which were starved for 18 hours and injected with adrenalin 1.9 γ /kilo 3 times in intervals of 10 minutes. The maximum rise of the blood sugar averaged 49% in this control group. The reaction was twice as great (average rise 97.9%) when this adrenalin experiment was repeated during the last half hour of an hour experiment in which the rabbits inhaled 7% oxygen. If, however, this period of anoxia was more prolonged (2 hours) and the same amount of adrenalin was

* Aided by a grant from the John and Mary R. Markle Foundation.

¹ Coffee, Ann, and Gellhorn, E., *Proc. Am. Physiol. Soc.*, Toronto, 1939, p. 51.

² McQuarrie, I., and Ziegler, M. R., *PROC. SOC. EXP. BIOL. AND MED.*, 1938,

administered during the last half hour of inhalation of 7% oxygen it no longer elicited a hyperglycemia (average rise of blood sugar 3%). It is interesting to note that even after readmission of air the glycogenolytic action of adrenalin remains reduced for considerable periods of time. Eight experiments were carried out in which adrenalin was reinjected $\frac{1}{2}$, 1, $1\frac{1}{2}$, and 4 hours, respectively, after the end of the anoxia period. In only one experiment was the response normal, but was greatly reduced in the other experiments. The average rise in blood sugar after adrenalin was 17.6%, as compared with 49% under control conditions.

Further studies showed that adrenalin loses its glycogenolytic effect on the liver during prolonged anoxia in spite of the fact that the liver still contains considerable amounts of glycogen. Seven experiments were performed in which adrenalin was given during the last half hour of a 2-hour period of 7% O₂ inhalation producing a rise of blood sugar of only 4.2% on the average, although the average glycogen content of the liver determined immediately at the end of the anoxia period was 3.3%. It seems to be of principal interest to state that an anaerobic reaction (glycogenolysis produced by adrenalin) is greatly modified by a moderate degree of anoxia. The influence of anoxia on the effect of other glycogenolytic factors is being studied at the present time.

Conclusions. 1. Short periods of moderate anoxia (7% oxygen) act antagonistically to insulin hypoglycemia, whereas prolonged periods (2 hours) aggravate hypoglycemia. 2. The glycogenolytic action of adrenalin is increased during a short period of anoxia but greatly decreased or lost completely after a prolonged period of anoxia. 3. This loss of the glycogenolytic effect of adrenalin on the liver is not due to a depletion of the glycogen reserves of the liver.

10671 P

Metabolism of N-Alkyl Derivatives of Amino Acids.

HERBERT E. CARTER AND PHILIP HANDLER. (Introduced by William C. Rose.)

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The metabolism of certain N-alkyl derivatives of phenylalanine, valine, leucine, and isoleucine has been studied by the methods of Rose and coworkers.¹ Young white rats were fed an adequate diet containing carbohydrate, fat, inorganic salts, vitamins, and a mixture of purified amino acids. In the diets of the experimental animals the amino acid being studied was replaced by varying amounts of the corresponding N-alkyl derivatives. The growth of the rats was followed over a 28-day period.

The results of these studies are shown in Table I.

These data and the results of other investigators with the N-alkyl derivatives of histidine,² tryptophane,^{3, 4, 5} methionine,⁶ lysine,⁷ and cystine⁸ may be summarized as follows:

1. The N-methyl derivatives of the *d*- forms of the essential amino acids are not utilized by the white rat for growth purposes.

2. With the exception of cystine the N-methyl derivative of the *l*- form of the amino acid has the same nutritive value as the corresponding unmethylated *d*- form. Thus both the *d*- form¹ and the N-methyl derivative of the *l*- form of phenylalanine, methionine, tryptophane, and histidine support growth; while the *d*- form,¹ and

TABLE I.

N-Alkyl Amino Acid	Supports Growth
N-methyl- <i>d</i> -phenylalanine	—
N-methyl- <i>l</i> -phenylalanine	+
N-ethyl- <i>dl</i> -phenylalanine	—
N,N-dimethyl- <i>dl</i> -phenylalanine	—
N-methyl- <i>dl</i> -valine	—
N-methyl- <i>dl</i> -leucine	—
N-methyl- <i>dl</i> -isoleucine	—

¹ Rose, W. C., *Science*, 1937, **86**, 298.

² Fishman, J. B., and White, A., *J. Biol. Chem.*, 1936, **113**, 175.

³ Gordon, W. G., and Jackson, R. J., *J. Biol. Chem.*, 1935, **110**, 151.

⁴ Gordon, W. G., *J. Biol. Chem.*, 1938, **123**, xliii.

⁵ Kyu-sui, C., *Z. physiol. Chem.*, 1938, **257**, 12.

⁶ Patterson, W. I., Dyer, H. M., and du Vigneaud, V., *J. Biol. Chem.*, 1936, **116**, 277.

⁷ Gordon, W. G., *J. Biol. Chem.*, 1939, **127**, 487.

⁸ Kies, M., Dyer, H. M., and du Vigneaud, V., *J. Biol. Chem.*, 1939, **128**, 207.

the N-methyl derivative of the *l*- form of lysine, valine, leucine, and isoleucine fail to do so. It is interesting to note that whereas the N-methyl derivative of *l*-phenylalanine is utilized by the rat neither the N-ethyl nor the N,N-dimethyl derivative supports growth.

10672 P

Serological Classification of *C. diphtheriae*.

R. H. P. SIA AND C. H. HUANG.

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The recent introduction of the serological classification of *beta* hemolytic streptococci by Griffith¹ has helped greatly the epidemiological studies of scarlet fever.^{1, 2, 3} Classification of *C. diphtheriae* has been found of similar value by Durand and Guerin,⁴ and Eagleton and Baxter.⁵ The former described 5 distinct agglutinable types and the latter, 10. No standard serological classification, however, has as yet been universally adopted. Recently, Robinson and Peeney⁶ working with *gravis* strains distinguished 5 serological types among this group.

The tube method of agglutination was employed by previous workers. In dealing with the large number of cultures which an epidemiological study will necessarily involve, the tube-method would be time-consuming and a relatively large amount of material would be required. Besides, technical difficulties, such as rapidly settling cultures, autoagglutinable and granular strains may frequently be encountered. To obviate some of these difficulties, we have made use of the slide-agglutination technic as introduced by Coca⁷ for cholera examination, and later utilized by Krumwiede^{8, 9} for the identification of meningococcus and typhoid-paratyphoid organisms, and by Griffith¹ for the serological classification of *beta* hemolytic

¹ Griffith, F., *J. Hyg.*, 1934, **34**, 542.

² Swift, H. F., Lancefield, R. C., and Goodner, K., *Am. J. M. Sc.*, 1935, **190**, 445.

³ Wu, C. J., and Sia, R. H. P., *Chinese Med. J.*, 1939, **55**, 150.

⁴ Durand, P., and Guerin, J., *C. R. Soc. biol.*, 1921, **84**, 980.

⁵ Eagleton, A. J., and Baxter, E. M., *J. Hyg.*, 1923, **22**, 107.

⁶ Robinson, D. T., and Peeney, A. L. P., *J. Path.*, 1936, **43**, 403.

⁷ Coca, A. F., *Bull. Manila Med. Soc.*, 1910, **2**, No. 1.

⁸ Krumwiede, C., *J. A. M. A.*, 1917, **69**, 358.

⁹ Krumwiede, C., *J. Inf. Dis.*, 1918, **23**, 275.

streptococci. We have found it to be simple, rapid, economical and satisfactory.

In the absence of standard type-cultures, the production of type-agglutinating sera was necessarily slow. Immunization of rabbits was first carried out with several strains of diphtheric bacilli chosen at random. Other strains found not to be agglutinated by these sera were used to produce new sera. In this way, 10 different types have been obtained.

Typing was done by placing drops of a thick bacterial suspension on a slide; then with each drop, a loopful of one of the agglutinating sera was mixed. With the aid of a hand-lens, agglutination in one of the drops could be observed immediately.

By this method, 95 strains of virulent diphtheric bacilli isolated from clinical cases of diphtheria treated in this hospital during the last 2 years have been classified as follows:

(1) Type D30	23 strains	(5) Type D14	10 strains
(2) " D25	18 "	(6) " D40	8 "
(3) " D41	16 "	(7) " 6287	5 "
(4) " 12190	12 "	(8-10) " D15, D43, 1382,	1 strain
			each

Type D40 and 6287 strains showed slight cross-agglutination. However, the type-specificity of all the sera has been confirmed by the reciprocal absorption-tests.

Summary. For serological classification of *C. diphtheriae* the slide-agglutination technic has been used. Of 95 strains obtained locally from clinical cases, 92 fell into 7 distinct serological types.

10673

Experimental Trichophytid in Guinea Pigs.*

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The term "trichophytid" was introduced by Bloch to designate a generalized eruption occurring during the course of a localized trichophytosis. It has also been used to designate localized skin lesions

* Aided by a grant from the Graduate School of the University of Minnesota. Assistance in the preparation of this material was furnished by the personnel of Works Progress Administration, Official Project No. 665-71-3-69, Subproject No. 274.

at a distance, and different, from a primary trichophytosis. These reactions have been thought to be due to a blood-borne distribution of either the parasite or its products to the skin, which has become sensitized as a result of the primary infection. This theory is based almost entirely upon clinical observations. Although a number of workers have reported on experimental trichophytosis, and on the occurrence of allergic sensitization in the experimental disease,¹ I have been unable to find any reports of experimental production of a generalized eruption in animals, comparable to human trichophytid.

The experiments here reported have been carried on with a single strain of *Trichophyton mentagrophytes* (= *Tr. gypseum*) typical in all respects, isolated from a human infection of the nape which was probably contracted from a horse. Guinea pigs were inoculated by scarifying some of the fungus into the skin of the abdomen (previously depilated by barium sulphide) with sandpaper. Signs of infection were noted on the fourth or fifth day; the lesions extended a centimeter or two beyond the scarified area, and reached their maximum development about the tenth day; they were usually completely healed after 4 to 5 weeks.

To provoke an allergic reaction in the infected guinea pigs, they have been reinoculated with live spores, or with cell sap expressed from the mycelium, or with crude polysaccharide extracted from the mycelium. Spore suspensions were obtained by washing the surface of large (25 mm) Sabouraud agar slant cultures with physiological salt solution. Cell sap was obtained by collecting the mycelium from 7-day-old cultures in Sabouraud's liquid medium, squeezing it as dry as possible, mincing it finely by repeated passage through a food chopper, and expressing the sap by means of a hydraulic press. The liquid so obtained was passed through a Seitz filter. The remaining mycelium, now nearly dry, and weighing about 150 g, was suspended in a liter of 1% NaOH solution and heated just to boiling. Hydrochloric acid was added to the alkaline extract (separated from the mycelium) until a voluminous precipitate formed. After filtering, the liquid was added to 10 volumes of alcohol. The resulting precipitate was collected, dissolved in 100 cc of water. Crystals of trichloroacetic acid were added until no further cloudiness appeared. After removing this precipitate by filtering, the remaining liquid was neutralized and added to 10 volumes of alcohol. The resulting precipitate was collected and dried. This precipitate is called crude polysaccharide.

¹ This literature has recently been reviewed by De Lamater, E. D., and Benham, R. W., *J. Invest. Dermatol.*, 1938, **1**, 451, 469.

Twenty-two infected guinea pigs have been reinoculated intraperitoneally with live spores, in doses of either one-half or one agar slant, at intervals from 10 to 52 days after the primary scarification. All of these have exhibited some degree of reaction, but sensitivity is greatest at the time when the primary lesion has nearly, but not entirely healed, *i. e.*, about 25 to 30 days. Such animals show, 24 hours after the administration of the "shock dose," a marked erythema observed on all exposed areas of the skin (the depilated area of the primary lesion, the paws, ears, scrotum, nose and lips). In 4 cases the animals were completely depilated posterior to the shoulders. These showed a general erythema, most marked on the ventral surface, but visible on the back when compared with control infected animals, similarly depilated, which had not been reinoculated. In another 24 hours, desquamation begins. This is at first most marked at the site of the primary lesion, but extends beyond it; in depilated animals it is observed over the entire ventral surface (Fig. 1). Desquamation of the back has not been observed. Desquamation is complete in 3 to 5 days. In from 2 to 5 days there occurs desquamation of the paws, the skin peeling off in large plaques (Fig. 2). This may be completed in 24 hours, so that if the animals are not carefully watched it could be overlooked. Simultaneous with the peeling of the paws, the ears become covered with fine scales, which may persist for a week. One animal showed marked scaling of the face, beginning about the nose; it may have had an accidental primary lesion about the nose.

Animals inoculated earlier or later than the period of maximum sensitivity have all reacted to a lesser degree. In some the skin reac-

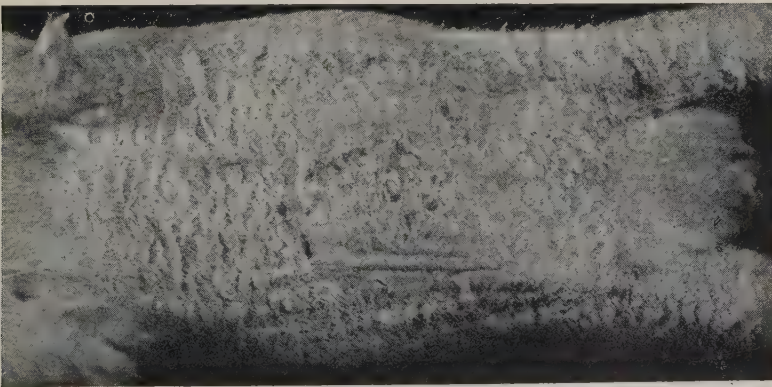


FIG. 1.

Generalized desquamation following intraperitoneal injection of "shock dose" of *Tr. mentagrophytes* into sensitized guinea pig.



FIG. 2.

Desquamation of paws following intraperitoneal injection of *Tr. mentagrophytes* into sensitized guinea pig.

tion was limited to the area of the primary infection and the paws or ears. Fourteen noninfected animals inoculated intraperitoneally with live spores have failed to show skin reactions of any sort. Four of these were completely depilated posterior to the shoulders before inoculation.

Four sensitized guinea pigs were given 5 cc of sterile cell sap intraperitoneally. They reacted in a manner identical with those which had been reinoculated with live spores. In 2 depilated animals complete desquamation of the ventral surface was noted. Peeling of the paws and scaling of the ears occurred in all. One animal given 0.2 cc of cell sap intracutaneously on the back showed a local area of redness at the site of injection, and desquamation of the primary lesion (which had healed), but no general reaction.

Two guinea pigs were given 50 mg of polysaccharide intraperitoneally. They showed general erythema followed by desquamation precisely as in those reinoculated with the live spores. Three additional animals received 10 mg of polysaccharide subcutaneously, and showed similar general reactions. Another, given 2 mg intracutaneously on the back, showed an intense local reaction with central necrosis, and general erythema, followed by desquamation of the paws, ears, and abdomen.

Two depilated noninfected controls were inoculated intraperitoneally with 5 cc of cell sap, and 2 others with 50 mg of polysaccharide, without any observable reaction. Six animals which had received a primary injection of live spores intraperitoneally were reinoculated in the same manner after 3 to 4 weeks. They showed no skin reactions.

Two sensitized guinea pigs which had reacted strongly to an intraperitoneal inoculation of live spores, were reinoculated with live spores after 3 weeks, and again reacted in a manner similar to the first reaction. Four animals which had reacted to intra- or subcutaneous injections of polysaccharide reacted again to live spores after a 3-week interval. One animal which had reacted to an intraperitoneal injection of cell sap, reacted again to an injection of live spores a month later; 2 months later, *i. e.*, 4 months after the initial scarification, it reacted to another injection of live spores by desquamation of the area of the original infection, but showed no generalized reaction.

I have looked for fungi in scales from the paws and ears and from the abdomen distant from the primary lesion without success in 6 cases. Cultures of scales from these animals also failed to show the presence of *Tr. mentagrophytes*. Sections of skin from paws and ears stained by the Gram-Weigert method have failed to reveal any fungi in 10 cases. Blood cultures have been made from 4 sensitized animals at intervals from 4 to 48 hours after intraperitoneal inoculation of live spores. They remained sterile.

In nearly all animals which developed trichophytid, enlargement of the scrotum was a marked feature. This was due in part to congestion and edema of the skin, but with those animals which were given live spores intraperitoneally, in the majority of cases palpable nodules developed in the testes after 4 to 7 days. These were found on autopsy to be confined to the surface of the testis, and to be part of a general peritoneal reaction, nodules occurring also in the omentum and on the parietal peritoneum. Similar but obviously lesser lesions were found in non-sensitized animals which had been inoculated intraperitoneally. These abdominal lesions are being studied further.

Summary. Guinea pigs which had recovered from infection with *Trichophyton mentagrophytes* reacted to intraperitoneal injections of live spores, cell sap, or polysaccharide by generalized erythema of the skin followed by desquamation. This condition is considered identical with trichophytid in human cases. No organisms could be found in the allergic lesions. These experiments strongly support the theory that trichophytid is an allergic response of the skin to substances in solution distributed to it by the blood.

Inactivation of Toxins of *Staphylococcus aureus* and *Clostridium welchii* *in vitro* by Sulfanilamide.*

CHARLES M. CARPENTER AND GERALD M. BARBOUR. (Introduced by George Packer Berry.)

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Recently, we^{1, 2} demonstrated that sulfanilamide prevents the death of mice which have been injected intraabdominally with lethal amounts of gonococcal "toxin", and that this "toxin" can be inactivated *in vitro* by exposure to the drug for several hours at 37°C. It was later observed³ that a toxin obtained from a single strain of *Staphylococcus aureus* became non-toxic immediately after contact with sulfanilamide *in vitro*. The present paper records the results of a similar study with the toxins of other strains of *Staphylococcus aureus* and the toxin of *Clostridium welchii*. Osgood and Powell⁴ have reported, "Sulfanilamide in concentrations of 1:1,000 or less does not inactivate *in vitro* significant amounts of the hemotoxins of . . . hemolytic *Staphylococcus aureus* . . . or *B. perfringens*." This finding is not in accord with the results which we have obtained during the last year.

The toxins from 3 hemolytic strains of *Staphylococcus aureus* were investigated. Two of the strains were recovered in our laboratories from purulent discharges, one from otitis media and the other from a furuncle. The third strain, received from Dr. C. E. Dolman, was isolated from the nasal mucosa of a patient with small boils in and about the nose. The different lots of toxin were prepared according to the technic of Dolman.⁵ A 36-hour culture of the organism grown on semi-solid agar was combined with Douglas' broth and filtered through cheese-cloth and filter paper. It was then centrifugalized, and the supernate, which constituted the toxin, was removed. Merthiolate was added to give a final concentration of 1:8,000.

* This study was financed in part by a grant from the Parke, Davis & Company. The sulfanilamide was supplied by the Winthrop Chemical Company.

¹ Carpenter, C. M., Hawley, P. L., and Barbour, G. M., *Science*, 1938, **88**, 530.

² Carpenter, C. M., Barbour, G. M., and Hawley, P. L., *J. Bact.*, 1938, **36**, 280.

³ Carpenter, C. M., Barbour, G. M., and Hawley, P. L., *J. Pediatrics*, 1939, **14**, 116.

⁴ Osgood, E. E., and Powell, H. M., *Proc. Soc. Exp. Biol. and Med.*, 1938, **39**, 37.

⁵ Dolman, C. E., *Canad. Public Health J.*, 1932, **23**, 125.

The MLD of each lot of toxin was determined for 20 g Rockland mice and was found to range from 0.1 to 0.2 cc. Sulfanilamide (in 0.85% sodium-chloride solution) in dilutions of from 1:33 to 1:1,000 was mixed with the toxin in a ratio of 1 cc of the dilution of the drug employed to 1 MLD of the toxin. The mixture was shaken and immediately injected intraabdominally into mice. A total of 825 mice was injected, 195 of which were controls. From 89 to 97% of 630 mice receiving the "inactivated" toxin lived, the percentage varying inversely with the concentration of the sulfanilamide used for inactivation. None of the 195 control mice survived (Table I). The work extended over a period of several months, during which time several different lots of toxin were used.

The inactivation of staphylococcal toxin by sulfanilamide *in vitro* prompted an investigation of the *in vivo* effects of the drug. The compound was injected intraabdominally into mice in doses ranging from 3 to 30 mg from 15 minutes to 3 hours prior to the intraabdominal administration of 1 MLD of the toxin. In this experiment, 144, or 60%, of 240 mice survived. When a single dose of 30 mg of the drug was similarly injected immediately after the administration of the toxin, 16, or 27% of 60 mice lived. Only 16, or 10% of 160 mice survived when sulfanilamide was administered in 2 doses, 10 mg immediately after the toxin and 20 mg 5 hours later.

Employing the procedure outlined for the *in vitro* inactivation of staphylococcal toxin by sulfanilamide, a similar study was made of the action of the drug on the toxin of *Clostridium welchii*.† The MLD for 20 g mice was determined to be 0.08 cc. An area of necrosis

TABLE I.
Intraabdominal Injection of Mice with Staphylococcal Toxin Inactivated *in Vitro* by Sulfanilamide.

Group	Dil. of sulfanilamide in 0.85% NaCl solution	Mice injected with toxin-sulfanilamide mixture			Mice injected with toxin only		Difference* S.E.D.M.
		No. injected	No. survived	% survived	No. injected	No. survived	
1	1:33	120	107	89.2	30	0	9.6
2	1:50	180	160	88.9	45	0	11.7
3	1:66	100	89	89.0	25	0	8.8
4	1:100	60	57	95.0	20	0	8.4
5	1:500	60	58	96.6	20	0	8.9
6	1:1000	110	107	97.3	55	0	13.5
Total		630	578	91.7	195	0	24.5

* Difference ÷ standard error of the difference of the means. The odds against the findings being due to chance approach infinity.

† The toxin of *Clostridium welchii* (perfringens-044692-B) was supplied by Parke, Davis & Company.

TABLE II.
Intraabdominal Injection of Mice with the Toxin of *Clostridium welchii* Inactivated *in Vitro* by Sulfanilamide.

Group	Dil. of sulfanilamide in 0.85% NaCl solution	Mice injected with toxin-sulf. mixture		Mice injected with toxin only		Difference* S.E.D.M.
		No. injected	No. survived	No. injected	No. survived	
1	1:33	20	15	5	0	
2	1:100	20	16	5	1	
3	1:500	20	15	5	1	
4	1:500	20	18	5	0	
5	1:1000	20	19	5	0	
6	1:1000	20	18	5	0	
Total		120	101 (84.2%)	30	2 (6.7%)	8.2

*Difference \div standard error of the difference of the means. The odds against the findings being due to chance approach infinity.

always developed at the site of injection. Findings at autopsy were usually generalized edema, subcutaneous hemorrhage, and focal areas of necrosis in the kidneys and liver. The toxin was mixed *in vitro* with the same dilutions of sulfanilamide as those used to inactivate the staphylococcal toxin. The mixture was injected at once, either intraabdominally or intramuscularly into mice. Eighty-four percent of 120 mice injected intraabdominally (Table II) and 86% of 140 mice injected intramuscularly survived. One MLD of the toxin, when injected intraabdominally, killed 93% of 30 control mice within 5 days. When the toxin was injected intramuscularly into the thigh, 89% of 30 control mice died within 7 days.

The *in vitro* inactivation of the toxin of *Clostridium welchii* was followed by a study of the therapeutic effect of sulfanilamide on mice injected with the toxin. It was found that when 30 mg of sulfanilamide in one cc of 0.85% sodium chloride solution were administered intraabdominally within 60 hours following the intraabdominal injection of 1 MLD of the toxin, 100, or 83% of 120 mice survived, while 90% of 30 untreated control mice died within 5 days. When the compound was given within 60 hours after intramuscular injection of the toxin, 108, or 90% of 120 mice were protected, while 87% of 30 untreated control mice died within 7 days.

The nature of the toxin-sulfanilamide reaction has not been determined. It is at present under study. The fact that the toxins, neutralized by exposure to sulfanilamide, regain their toxicity after various periods of standing at room temperature, indicates that the reaction is, in part at least, reversible.

Summary. Toxins from 3 hemolytic strains of *Staphylococcus aureus* were inactivated by sulfanilamide *in vitro*. This was demon-

strated by the survival of 92% of 630 mice injected intraäbdominally with the sulfanilamide-toxin mixture and the death of all 195 controls which received toxin only. The *in vivo* action of sulfanilamide, administered either before or subsequent to the toxin, was less marked.

The toxin of *Clostridium welchii* was likewise inactivated *in vitro* by sulfanilamide. Eighty-four per cent of 120 and 86% of 140 mice, injected intraäbdominally and intramuscularly, respectively, with the toxin-sulfanilamide mixture survived. *In vivo*, sulfanilamide protected 83% of 120 mice, when administered after the intraäbdominal injection of the toxin, and 90% of 120 mice after intramuscular inoculation. Only 8% of 60 control mice, injected intraäbdominally, and 12% of 60 control mice, injected intramuscularly, lived.

10675 P

Effect of Petroleum Ether Extract of Mouse Carcasses as Solvent in Production of Sarcoma.*

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The production of sarcomata in mice may be influenced by the solvent used for the carcinogenic hydrocarbon.¹ Most experiments have been carried out with vegetable oils, lard, cholesterol or paraffin which are effective vehicles for 1:2:5:6-dibenzanthracene, 3:4-benzpyrene and methylcholanthrene. The increase in the incidence of skin tumors in mice following the application of mouse fat to the skin before tarring, as reported by Watson and Mellanby,² led us to investigate the effect of a petroleum ether extract of mouse tissues as a solvent for 3:4-benzpyrene in the production of connective tissue sarcoma.

The extract was prepared by refluxing fresh minced mouse carcasses, from which the stomach and intestines had been excised, with petroleum ether (maximum boiling point 50°C) for 16 hours. The petroleum ether was removed by distillation under reduced pressure.

* This investigation was aided by a grant from the International Cancer Research Foundation.

† Research Fellow, the National Cancer Institute.

1 Peacock, P. R., and Beck, S., *Brit. J. Exp. Path.*, 1938, **19**, 315.

2 Watson, A. F., and Mellanby, E., *Brit. J. Exp. Path.*, 1930, **11**, 311.

The resulting mixture was turbid, yellow and oily at 37°C. A gray-white, greasy, amorphous precipitate formed on cooling. The whole extract consisted largely of neutral fats and free fatty acids.

Sesame oil and colloidal solutions of 3:4-benzpyrene were also used. The colloidal material was prepared by the gelatin method of Boyland.³ Each injection, representing 0.25 mg benzpyrene, was made in the subcutaneous tissues.

Three groups of 50 C57 black mice received a single injection of 3:4-benzpyrene in the inguinal region dissolved in sesame oil, petroleum ether extract of mouse carcasses or as a colloidal solution. In a fourth group of 50 mice, each animal received a single injection of each of the 3 solutions. All of the mice were obtained from the Roscoe B. Jackson Memorial Laboratory and were 5 to 6 weeks old when the injections were made. They were observed once weekly. The time when a progressively growing mass was first noted was taken as the appearance time of the tumor. Surviving animals were killed 32 weeks after injection. All diagnoses were confirmed by histologic examination. The effective total method was applied to the results.⁴

One tumor appeared among the 44 mice that received only a single injection of benzpyrene in the petroleum ether extract of mouse carcasses (Table I). The mice that were given benzpyrene in sesame oil developed more tumors than did those receiving colloidal benzpyrene. In the group of mice that was injected with benzpyrene in each of the 3 solutions only one tumor appeared when the solvent was petroleum ether extract of mouse carcasses (Tables II and III). It is possible that the much higher incidence of tumors resulting from the sesame oil and colloidal solutions caused the death of the animals before they had the opportunity to develop sarcoma at the site of injection of the extract. The experience with the group that received benzpyrene in petroleum ether extract of mouse carcasses alone makes this explanation less probable.

Andervont⁵ reported a difference in sarcoma production of male and female C57 black mice injected with 1:2:5:6-dibenzanthracene and methylcholanthrene. This was most pronounced in the latent interval of tumor production. In our series the incidence of tumors in the sexes was not significantly altered when sesame oil was the solvent. More male mice developed sarcomata when colloidal benzpyrene was administered. The latent interval to production of sarcoma was approximately the same for males and females except

³ Boyland, E., *Lancet*, 1932, **2**, 1108.

⁴ Fieser, L. F., *Am. J. Cancer*, 1938, **34**, 37.

⁵ Andervont, H. B., *Pub. Health Rep.*, 1938, **53**, 1647.

TABLE I.
Sarcomata in C57 Black Mice Receiving a Single Injection of 0.25 mg 3:4-Benzpyrene in the Inguinal Region.

Weeks No. of mice	Character of injection	Sex	No.	a. No. of new tumors b. Accumulated % mice with tumors	10	12	14	16	18	20	22	24	26	28	30	Total No. tumors	Died or killed without tumors
46	Dissolved in .25 cc sesame oil	M	21	a 1 2 1 3 5 3 b 5 14 19 33 57 71									1	1		17	
		F	25	a 2 10 3 b 8 48 60								3		1		19	10
46	Colloidal solution 0.5 cc	M	20	a 1 3 3 3 1 b 5 20 35 50 55									1			12	
		F	26	a 1 2 1 2 3 1 b 4 11 15 23 30 38 42								2	2	1		11	23
44	Dissolved in .25 cc extract	M	17	a b												0	
		F	27	a b							1					1	43

TABLE II.
Sarcomata in C57 Black Mice Receiving 3 Separate Injections of 0.25 mg
3:4-Benzpyrene.

Time in weeks				10	12	14	16	18	20	22	24	26	28	30		
Character of injection	Sex	No.	a	No. of new tumors											Total No. tumors	
			b	Accumulated % of mice with tumors												
Dissolved in .25 cc sesame oil	M	19	a	3	3	4	1					1			12	
			b	16	32	53	58					63				26
	F	26	a	2	2	4	2	2	2						14	57%
			b	8	15	30	38	46	53							
Colloidal solution 5 cc	M	19	a	3	2	3	1	2	1						12	
			b	16	25	42	47	58	53							19
	F	26	a	1	2		1	2				1			7	41%
			b	4	11		15	23				27				
Dissolved in .25 cc extract	M	19	a												0	
			b													1
	F	26	a									1			1	2%
			b									4				

TABLE III.
Sex Distribution of Sarcomata in C57 Black Mice Receiving 3 Separate Injections
of 0.25 mg 3:4-Benzpyrene.
(Effective total—45; 19 male, 26 female.)

Mice	Male	Female	Total
Died without tumor	1	8	9
One tumor only	12	14	26
Sesame oil	6	10	16
Colloid	6	3	9
Extract	0	1	1
Two tumors	6	4	10

in the group receiving a single injection of colloidal benzpyrene. It is questionable whether the greatest difference, 3.1 weeks, is statistically significant.

Factors involved in the extreme reduction in the incidence of sarcoma following the injection of 3:4-benzpyrene in petroleum ether extract of mouse carcasses are being investigated.

10676

Valine and Isovaleric Acid Show Positive Influence upon Hemoglobin Production in Anemia Due to Blood Loss.

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The experiments tabulated below give evidence that the anemic dog can use valine to aid in the building of new hemoglobin. Moreover the dog can use the optical isomer (antipode) as well as the naturally occurring amino acid (*d*-form). It has been reported¹ that histidine and phenylalanine react as does valine. It is generally believed that before the optical isomer can be utilized in the body it must be deaminized and perhaps be recast in the natural form. A variety of mechanisms could come into play to explain the observations relating to the optical isomers.² When these observations were reviewed with Dr. Rose, he raised the question as to the related fatty acids. The experiments below indicate that isovaleric acid can at times be used by the anemic dog in building new hemoglobin under the conditions of these experiments.

The technical procedures related to these experiments have been fully described³ and the preparation of the basal ration (salmon bread) has been reviewed. Pure crystalline amino acids were used in these experiments. These anemic dogs are standardized over a period of years and their response in hemoglobin production to various factors well established. The control figures are given in Table I not only for the standard feeding of liver but for iron in 40 mg doses per day. The standard salmon bread used in all but 2 experiments contained 3 mg Fe per 100 g as fed. The 2 experiments under *d*-valine, Table I (Dogs 35-7 and 32-5) were carried out with a salmon bread which contained approximately 9 mg Fe per 100 g and this accounts for the high output on the salmon bread alone. Under these experimental conditions we consider a production of 10 g hemoglobin over and above the control basal bread output to be significant.

Table I shows that the natural *d*-form of valine may influence hemoglobin production. In 3 experiments the output of hemoglobin

¹ Whipple, G. H., and Robscheit-Robbins, F. S., *Proc. Soc. Exp. Biol. and Med.*, 1937, **36**, 629.

² *Ann. Rev. of Biochemistry*, 1936, 253.

³ Whipple, G. H., and Robscheit-Robbins, F. S., *Am. J. Physiol.*, 1936, **115**, 651.

TABLE I.
Valine and Isovaleric Acid Influence Hemoglobin Production in Anemia.

Dog No.	Valine and isovaleric acid fed		Control net hemoglobin output per 2 wk		
	Daily dose, g	Hemoglobin net output per 2 wk, g	Iron 40 mg daily-oral, g	Liver 300 g daily-oral, g	Basal bread ration alone, g
Valine, d-form (natural)					
35-7	1	33	61	82	22
35-2	1	35	48	104	4
32-5	1	3	69	86	34
29-326	1	4	56	84	4
35-4	1	26	56	89	4
Valine, l-form (optical isomer)					
35-7	1	21	51	82	4
36-11	1	12	65	66	4
33-14	1	7	58	72	4
32-5	1	3	58	98	4
Isovaleric Acid					
34-148	1	11	52	85	4
35-2	1	10	48	104	4
35-7	1	32	51	82	4
35-4	1	53	56	89	12

ranges from 26 to 35 g above the control levels. Two other experiments are negative (3 to 4 g hemoglobin).

Valine (optical isomer or *l*-form) is a little less potent. Two experiments may be listed as positive (12 and 21 g hemoglobin net output) and 2 as negative (3 and 7 g hemoglobin net output).

Isovaleric acid (Table I) if anything is a little more potent than *l*-valine but we would not stress these differences—at least the general trend is similar. Two experiments are on the border line (10 and 11 g hemoglobin net output) but 2 others show significant outputs of hemoglobin (32 and 53 g).

In a considerable number of experiments with a variety of amino acids we have observed that in the majority of experiments there is a positive response—that is more than 10 g hemoglobin produced during the test period over and above the bread base line. There are also frankly negative experiments as recorded in Table I.

It is difficult to give a satisfactory explanation for the observed facts but we prefer the following argument. The standard dog ingests with his food one gram a day of the given amino acid which is absorbed together with a great mixture of amino acids coming from the digested protein in the standard salmon bread (wheat protein and canned salmon muscle). To make extra hemoglobin the dog must supply the other amino acids to supplement the specific amino acid given and these accessory amino acids must be derived from the food

intake (standard bread), from body stores, or from protein catabolism. We assume that in this long continued anemia there is a stimulus for the dog to utilize all available material to make new and badly needed hemoglobin. The added amino acid may accelerate the flow of other amino acids in the direction of globin production which globin we assume is the limiting factor in certain experiments. When the amino acid feeding experiments are frankly negative we may assume that one or more of the many supplements which the body must add are not available during that particular period and hemoglobin synthesis fails.

If the animal can break up certain amino acids and recombine the mangled remains⁴ to form other amino acids and body protein, there is no reason why this same reaction can not take place in the rapid production of the protein hemoglobin in experimental anemia. Therefore it is reasonable to test various simple substances closely related to amino acids to ascertain whether the dog can utilize these substances in hemoglobin construction. Isovaleric acid appears to qualify in this respect and we plan to continue a study of related compounds.

10677 P

Some Effects of Feeding Thyroid to Immature Fishes (*Platypoecilus*).

CLIFFORD GROBSTEIN AND ALBERT W. BELLAMY. (Introduced by
Gordon H. Ball.)

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Striking effects have been obtained by feeding desiccated mammalian thyroid (Parke Davis & Co.) to sexually immature poeciliid fishes of the genus *Platypoecilus*. The details of the response are now under investigation but its general nature may be briefly described.

Control and experimental animals were secured by dividing single progenies and placing them in adjacent tanks. Both groups received routine laboratory feeding—ground liver and young brine shrimp on alternate days. In addition, the experimental groups each morning throughout the experiment received a pinch of thyroid powder scat-

⁴ Schoenheimer, R., Ratner, S., and Rittenberg, D., *J. Biol. Chem.*, 1939, **127**, 333.

TABLE I.
Summary of Data on Isolation Age.

Progeny	Treatment	No. of animals	No. of maturing males	Avg isolation age (days)	Range in isolation age (days)	
<i>Variatus</i>						
853f	Lot 1	Control	17	6	88.2	64-102
	Lot 2	Thyroid-fed	18	10	39.3	35-53
853i	Lot 1	Control	13	5	84.8	57-166
	Lot 2	Thyroid-fed	14	3	46.3	39-52
	Lot 3	" "	13	4	45.8	39-52
<i>Maculatus</i>						
914c	Lot 1	Control	21	10	189.7	126-215
	Lot 2	Thyroid-fed	21	8	99.1	86-126

tered on the surface of the water. They were observed to feed readily on this powder.

One hundred and thirty-seven fish derived from 7 progenies have been fed thyroid. The results obtained from the study of 3 of these progenies, 2 of *P. variatus* and one of *P. maculatus*, will be described as typical. The essential data on these 3 progenies are summarized in Table I.

All thyroid-fed animals developed marked exophthalmos. After several months of continued feeding the protrusion of the cornea became so great that the eyes stood out globe-like on either side of the head. The protrusion still was clearly apparent after several months of preservation in formalin. In practically all cases exophthalmos was bilateral and equal. In only one of 2 cases was there some degree of inequality.

Both size and body proportions were affected by thyroid feeding. Apart from one exceptional group the experimental fish were always considerably smaller than the controls. In 853i, for instance, 5 weeks after the initiation of thyroid feeding the average body length of the control group was approximately 25% greater than that of the experimental groups. In addition to being smaller thyroid-fed fish are decidedly longer in proportion to their depth with all fins conspicuously elongate.

Sex maturation in treated males (and probably also in females) is speeded if beginning of differentiation of the anal fin into its definitive sex form may be accepted as a criterion. It is part of the routine of this laboratory to isolate sexually immature fish at the first sign of anal fin changes leading to the development of the gonopod or intromittent organ characteristic of the male. This may be referred to as the "isolation age." (See Table I.) Seventeen thyroid-fed *variatus* males were isolated at an average age of 42.4 days while 11 control *variatus* males were isolated at an average age of 86.6

days. In the *maculatus* progeny the average isolation age of 8 thyroid-fed males was 99.1 days, that of 10 control males 189.7 days. The sharp difference in the isolation ages shown by *maculatus* and *variatus* appears to be characteristic of these species (Bellamy—unpublished data). There seems to be little doubt that thyroid feeding results in precocious maturation in both species tested.

Besides being precocious, differentiation of the gonopod in thyroid-fed males is atypical. In the normal male the third, fourth, and fifth anal rays grow out to nearly twice the length of the sixth and seventh and undergo a closely integrated differentiation. In the thyroid-fed male, however, the sixth and seventh rays also participate in the elongation process and the typical relations of the rays are upset. Subsequent differentiation is incomplete and distorted. Description of the details of the process must be postponed for a later publication.

Summary. Thyroid-feeding of immature *Platyopocilus maculatus* and *P. variatus* resulted in exophthalmos, decreased growth rate and altered body proportions, in addition to precocious sex maturation as indicated by the early but atypical differentiation of the male gonopod.

10678

Cultivation of Various Species of Trypanosomes in the Developing Chick Embryo.

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One of the strains of trypanosomes (*T. rhodesiense*) maintained in this laboratory throughout a period of several years became arsenic-fast for some unknown cause. Cross-transfer to various kinds of mammalian hosts failed to correct this abnormality, hence the idea occurred to us to attempt cultivation of the organism. Since cultivation in artificial media was unsatisfactory, we turned next to the chick embryo as a further possibility.

Early in the work we used the commonly employed window technic but soon found that a simplified method was entirely adequate. Eggs were incubated for 8 or 10 days prior to inoculation. By means of a sterile dissecting needle, two small holes were made through the shell previously cleaned with alcohol. One hole was made into the

air sac and the other immediately over the embryo. The inoculum consisted of rat blood, highly infected with trypanosomes obtained aseptically and diluted with an equal volume of 0.9% NaCl solution. A 26-gauge needle attached to a syringe was then inserted into the allantoic cavity by placing the needle parallel to and immediately under the chorio-allantoic membrane. The volume of inoculum for each egg was usually approximately 0.5 cc. After withdrawing the needle, the holes in the shell were closed by small drops of melted paraffin. A heavy infection in embryo blood was regularly obtained which was the cause of death of the embryos in 4 or 5 days.

Subcultures were made by transferring diluted embryo blood obtained aseptically on the fifth day. By this technic this strain was maintained in developing chick embryos for 8 generations in 41 days. Neither change in virulence nor arsenic-fastness occurred in this strain of trypanosomes during this period of observation.

Since *T. rhodesiense* was so easily cultivated in the chick embryo, it seemed appropriate to attempt cultivation of other species of trypanosomes. On trial it was found that other species, *viz.*, *T. equiperdum*, *T. brucei*, *T. evansi* and *T. hippicum*, were equally readily cultivated for 15 days and maintained their normal virulence for rats. There appeared to be no obvious reason why these strains could not have been cultivated indefinitely had there been any object for such a continuation. *T. lewisi*, on the other hand, was cultivated, but not very satisfactorily, because of its very slow growth.

None of these species of trypanosomes was infective for the hatched chick.

Conclusions. The following species of trypanosomes were successfully cultivated in the developing chick embryo: *rhodesiense*, *equiperdum*, *brucei*, *evansi* and *hippicum*. *T. lewisi* was cultivated but not very satisfactorily. No change in virulence of these organisms appeared during the time of cultivation. The strain of *T. rhodesiense* remained arsenic-fast throughout the period of 41 days of cultivation in the chick embryo.

10679

Effects of Anesthetics on the Response of Submaxillary and Pancreatic Glands to Prostigmine and Physostigmine.

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In an analysis of the nervous secretory mechanism of a gland it is appropriate to use drugs which may excite or depress nerve endings. In this investigation prostigmine and physostigmine were selected. Prostigmine was selected because its effect on pancreatic secretion and its relative effect on pancreatic and salivary secretion has not been determined. Physostigmine was selected because, although it is known to stimulate pancreatic^{1, 3} and salivary² secretion, the relative action of the drug on the pancreatic and salivary glands has not been determined in the same animal. It was also desired to ascertain whether as the dosage of the drug was increased a "reversal" in the secretory response of the gland occurred, as had been shown to occur in the case of the action of acetyl-beta-methyl choline on the gastric glands.⁴

Dogs were anesthetized with either sodium pentobarbital (30 mg per kilo), chloralose (French, 100 mg per kilo), or paraldehyde (1.8 cc per kilo). The latter 2 anesthetics were employed because the former may have a pseudo-atropine action on the vagus.⁵

The pancreatic and submaxillary ducts were cannulated. A blood pressure record was made to ascertain the simultaneous effects of the drugs on the circulatory system. The pyloric sphincter was occluded to prevent the gastric secretion from entering the intestine. The drugs were injected intravenously. *Prostigmine* was given in doses ranging from 0.005 to 0.2 mg per kilo; *physostigmine* in doses ranging from 0.01 to 0.2 mg per kilo. The drugs were injected either at 30- or 60-minute intervals. A control secretion was always obtained and a basal flow was awaited before a second injection of a drug was made.

¹ Crittenden, P. J., and Ivy, A. C., *Am. J. Physiol.*, 1937, **118**, 724.

² Loewi, O., and Mansfield, G., *Arch. f. exp. Path. u. Pharmacol.*, 1909-10, **62**, 180.

³ Babkin, B. P., Herb, C. O., and Sergeyev, M. A., *Am. J. Physiol.*, 1938, **123**, 5.

⁴ Gray, J. S., and Ivy, A. C., *Am. J. Physiol.*, 1937, **120**, 705.

⁵ Linegar, C. R., Dille, J. M., and Koppanyi, T., *J. Pharm. and Exp. Therap.*, 1936, **58**, 128.

Pancreatic Secretion: Pentobarbital anesthesia. The following observations pertain to the results obtained on 11 animals. *Prostigmine* stimulated pancreatic secretion in all animals. The threshold dose was 0.005 mg per kilo. The maximal secretory response, which on the average amounted to 35 drops or 1.7 cc was obtained with 0.06 mg per kilo. Larger doses produced less and less secretion, so that with a dose of 0.2 mg per kilo only 17 drops or 0.8 cc of juice were obtained. *Physostigmine* stimulated pancreatic secretion. The threshold dose was 0.01 mg per kilo and the maximum secretory response was obtained usually with 0.2 mg per kilo, the average maximal output of secretion being 30.5 drops or 1.5 cc. A reversal of the response with larger doses could not be clearly established because larger doses were frequently fatal.

These results show that the pancreas is more sensitive to *prostigmine* than *physostigmine* and that a "reversal" of the secretory response may occur with large doses of prostigmine.

When the injections were made at one-hour intervals the results were the same, with the exception that they showed that the action of *prostigmine* on the pancreas lasted somewhat longer than 30 minutes.

Salivary Secretion: Pentobarbital anesthesia. The threshold dose of *prostigmine* was about 0.01 mg per kilo. The maximal secretory response occurred with a dose of 0.1 mg per kilo and average 7.5 cc in amount. With larger doses a "reversal" in the secretory response occurred as in the case of the pancreas. The threshold dose of *physostigmine* was about 0.03 mg per kilo and the maximal secretory response, average 1.3 cc, was obtained with 0.2 mg per kilo.

Thus, the submaxillary gland responds to *prostigmine* and *physostigmine* similarly to the pancreas, with the exception that the submaxillary gland produces a greater volume of secretion. The dosage differences are not due to differences in molecular concentration of the drugs.

Chloralose and paraldehyde anesthesia. Five animals were anesthetized with chloralose and 4 with paraldehyde. Both of these anesthetics raised the threshold dose and decreased the volume output of secretion in the case of each drug. More experiments were not performed with these anesthetics because their deleterious effects were obvious. For example, with chloralose anesthesia the maximal secretory response to *prostigmine* averaged only 3.6 cc instead of 7.5 cc under pentobarbital.

With equivalent doses, *prostigmine* had a greater effect on blood pressure (increases, as a rule) and heart rate (slows) regardless of

the anesthesia used, than *physostigmine*. This observation is contrary to that made by Aeschilman and Reinert⁶ on the frog and rabbit, but it supports the use of belladonna and its alkaloids when large doses of *prostigmine* are given in myasthenia gravis.⁷ The effects of *physostigmine* on blood pressure were more variable than those of *prostigmine*, but agree with the effects reported by Heathcote⁸ in that small doses usually cause a rise and large doses a fall. The effect of *prostigmine* on the heart rate of chloralosed dogs was most striking. With a dose of 0.05 mg per kilo and above, the pulse was markedly slowed and the pulse pressure was greatly increased, measuring from 70 to 90 mm Hg.

Only one point of interest will be discussed and that is in reference to the mechanism of the *prostigmine reversal*, which is of interest in relation to observations made previously on the response of the pancreas and stomach to acetyl choline.^{1, 3, 4} Chloralose affected the cardiac mechanism so that *prostigmine* caused a very large pulse pressure which indicated vagal stimulation. Chloralose also markedly decreased the response of the glands to *prostigmine*. This suggests that the "reversal" noted with pentobarbital anesthesia was due to a selective stimulation of the excitatory and inhibitory mechanisms of the gland, the larger doses affecting the inhibitory mechanism chiefly. Chloralose probably stimulated or sensitized the inhibitory mechanism of the gland and then *prostigmine* produced less secretion than when pentobarbital was the anesthetic. Koppanyi⁵

TABLE I.
Average Effect of Pancreatic Secretion in Drops.

Dose in mg per kilo		0.01	0.02	0.05	0.06	0.075	0.1	0.2
<i>Prostigmine</i>								
Pentobarbital	30'	2	9.5	22	35	19	17	12
"	60'		7.5	17		16.5	14	
Paraldehyde			0.0	2		7	18	
Chloralose		0.5	2	2.5		8	5	2
<i>Physostigmine</i>								
Pentobarbital	30'	0.5	1.6	7		7	12.5	30
"	60'			6		10	12	
Paraldehyde				2		1	4	
Chloralose				2		0.0	1.5	

This table shows (in drops) the average effects of various doses of *prostigmine* and *physostigmine* on pancreatic secretions in animals anesthetized with either sodium pentobarbital, paraldehyde, or chloralose. The "reversal" of effect on the secretion is marked following *prostigmine* when the animals are anesthetized with sodium pentobarbital and the drug injected at thirty-minute intervals.

⁶ Aeschilman, J. A., and Reinert, M., *J. Pharm. and Exp. Therap.*, 1931, **43**, 413.

⁷ Walker, M. B., *Proc. Roy. Soc. Med.*, 1935, **28**, 759.

⁸ Heathcote, R. St. A., *J. Pharm. and Exp. Therap.*, 1932, **44**, 95.

has presented evidence indicating that pentobarbital has an atropine-like action in the cardiac vagal mechanism. If it has a similar effect on the "parasympathetic" or vagal inhibitory mechanism of the pancreas (or submaxillary), then pentobarbital would favor *prostigmine* stimulation of secretion and also *physostigmine* stimulation, which it does; and, on the basis that chloralose has the opposite action (*i. e.*, to pentobarbital) on the inhibitory mechanism, less secretion would be obtained with both *prostigmine* and *physostigmine*, which was observed. The observed effects of paraldehyde, however, do not agree with the foregoing hypothesis because paraldehyde affected the secretory response to *prostigmine* and *physostigmine* like chloralose, but not the cardiac response, although the degree of cardiac slowing was similar.

Summary and Conclusions. *Prostigmine* and *physostigmine* were administered intravenously in doses ranging from 0.005 mg to 0.2 mg per kilo to dogs anesthetized with either sodium pentobarbital, chloralose, or paraldehyde. The effects on pancreatic and salivary secretion and on the blood pressure and heart rate were recorded.

1. In the lower doses *prostigmine* is a more potent excitant of pancreatic and submaxillary secretion than *physostigmine*. A "reversal" in the response of the pancreas occurred when the dose of *prostigmine* was increased above 0.06 mg per kilo, and of the submaxillary gland in doses above 0.1 mg per kilo.

2. Chloralose anesthesia markedly diminishes the secretory response of the pancreas and submaxillary secretion to *prostigmine* and *physostigmine*; the same is true of paraldehyde anesthesia.

10680

Determination of Vitamin C Nutrition by Means of a Skin Test. A Critical Evaluation.

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(Introduced by J. H. Musser.)

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Rotter^{1, 2} suggested that the state of vitamin C nutrition could be determined by means of a skin test in which 0.01 cc of a 1/400

¹ Rotter, H., *Nature*, 1937, **139**, 717.

² Rotter, H., *Klin. Wchnschr.*, 1938, **51**, 205.

normal solution of the dye, 2-6-dichlorphenol-indophenol, was injected intradermally and the time required for decolorization of the dye noted. He concluded that a decolorization time of more than 10 minutes indicated vitamin C deficiency; a time of 5 to 10 minutes, a normal state of nutrition; and a time of less than 5 minutes, saturation of the tissues with ascorbic acid. Portnoy and Wilkinson,³ in a study of 25 persons, found that the mean decolorization time of the intradermal test was 16.9 minutes when the level of ascorbic acid in the blood was subnormal (0.27-0.52 mg/100 cc), 7.5 minutes when the level in the blood was normal (0.72-1.3 mg/100 cc), and 2.3 minutes when the level had been raised to 1.32 to 2.0 mg/100 cc by saturating the individual with ascorbic acid. These results indicated an inverse relationship between the time required for decolorization of 2-6-dichlorphenol-indophenol by the skin and the amount of vitamin C in the blood.

Poncher and Stubenrauch,⁴ however, failed to substantiate these findings. In a study of 41 patients they found that the time of decolorization of the skin test averaged 5.8 minutes in 6 persons who had scurvy, 9.4 minutes in 9 individuals who had a subnormal level of vitamin C in the blood, and 7.6 minutes in 26 persons in whom the ascorbic acid in the blood was normal. Jetter⁵ likewise found the intradermal test an unsatisfactory index of vitamin C nutrition. He studied 50 females with active tuberculosis, and in all instances the decolorization time was between 2 and 10 minutes, although the ascorbic acid in the blood varied from 0.4 to over 1.39 mg/100 cc.

This study comprises 100 observations on 45 patients, a comparison being made of the amount of ascorbic acid in the blood and the time required for decolorization of 2-6-dichlorphenol-indophenol injected into the skin. Subjects who originally showed a subnormal amount of ascorbic acid in the blood were given 200 to 300 mg of vitamin C daily and retested at intervals until the level in the blood indicated saturation of the body.

The amount of ascorbic acid in the blood was determined by the method of Farmer and Abt.⁶ The skin test was performed according to the technic of Rotter, 0.01 cc of a solution of 2-6-dichlorphenol-indophenol containing 2.0 mg of the dye in 4.9 cc of distilled water being injected intradermally into the volar surface of the forearm. The dye was used either without sterilization, since it

³ Portnoy, B., and Wilkinson, J. F., *British M. J.*, 1938, **1**, 328.

⁴ Poncher, H. G., and Stubenrauch, C. H., *J. A. M. A.*, 1938, **111**, 302.

⁵ Jetter, W. W., *PROC. SOC. EXP. BIOL. AND MED.*, 1938, **39**, 169.

⁶ Farmer, C. J., and Abt, A. F., *PROC. SOC. EXP. BIOL. AND MED.*, 1936, **34**, 146.

TABLE I.

No. tests	Ascorbic acid Blood plasma (mg%)	Decolorization time-intradermal test (min)		
		Range	Mean	Standard deviation
26	0.00-0.69 (Subnormal)	2.0-14.75	8.69 \pm 0.383	2.89 \pm 0.271
42	0.70-1.29 (Normal)	2.0-17.50	6.93 \pm 0.322	3.10 \pm 0.228
32	1.30-1.99 (Saturated)	3.0-15.40	8.31 \pm 0.321	2.69 \pm 0.227

was found to be bacteria-free, or after passage through a Seitz filter, as suggested by Portnoy and Wilkinson.³ This latter manipulation did not affect the results. Since part of the discrepancy in the findings of various workers could have been due to the amount of dye injected intradermally, a device⁷ was employed which allowed delivery of exactly 0.01 cc from a tuberculin syringe. To avoid another possible cause of inconstant findings, that is, differences in blood flow due to the position of the extremity, the patient was always seated with the forearm resting slightly below the heart level on a table.

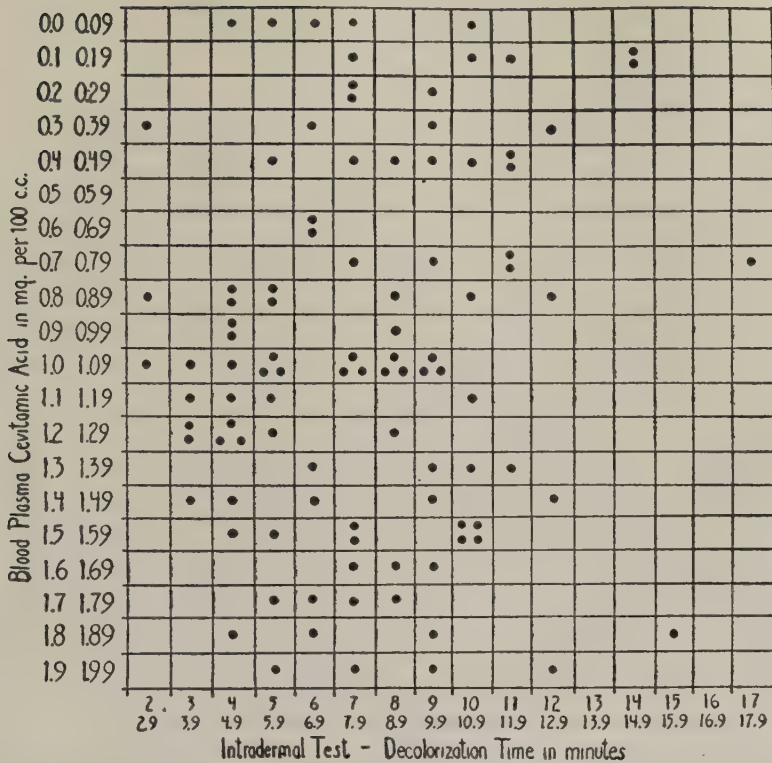
Table I shows the relation between the time of decolorization of the intradermal tests with 2-6-dichlorophenol-indophenol and the level of ascorbic acid in the blood plasma in 100 tests. It is evident that the time required for fading of the dye shows the same variation whether the amount of vitamin C in the blood was high or low. If the mean decolorization time of skin tests in the group with a normal quantity of ascorbic acid in the blood is compared with that of the group showing subnutrition, there is a difference of 2.76 ± 0.50 , which is statistically significant. However, if the mean decolorization time of the skin tests of the persons who had subnutrition is compared with that of the persons who were saturated, the difference is 0.379 ± 0.499 , obviously not statistically significant.

The complete lack of correlation between the decolorization time of the skin test with 2-6-dichlorophenol-indophenol and the content of ascorbic acid in the blood is shown in Fig. 1. The coefficient of correlation for the 100 tests was found to be -0.106 ± 0.067 . It is obvious that the skin test in its present form cannot be used as a substitute for examination of the blood in determining the state of vitamin C nutrition.

Examination of the data obtained in individuals who had repeated skin and blood tests at different levels of vitamin C nutrition (subnormal, normal and saturated) suggested that there might be some correlation in a given individual between these two determinations. Accordingly, for each of 9 patients who had had more than 5 tests, the percentage deviation of each determination from the mean for

⁷ Burch, G. E., *PROC. SOC. EXP. BIOL. AND MED.*, 1939, **40**, 676.

FIGURE ONE



that individual was calculated. The correlation coefficient was again not significant, being 0.293 ± 0.092 .

In order to estimate the magnitude of the error of a single intradermal test, one individual was given a series of 5 injections in the forearm every 15 minutes until a total of 25 injections was reached. The time of decolorization of the dye varied from 4.5 to 11.6 minutes with a mean of 8.9 ± 0.283 minutes. The standard deviation was 2.097 ± 0.199 . These figures correspond closely with those obtained in the study of the patients who showed vitamin C subnutrition or saturation.

There are a number of factors which might affect the variability of an intradermal test, especially when this test depends upon the quantity of reducing substances in the skin. Vitamin C is only one, and possibly not the most important, reducing agent present. Changes in the circulation and in the amount of oxygen available in the tissues might influence the rate of reduction of the dye. Variations in room temperature and in the position of the forearm could

be important in this regard. The lymphatic drainage of the forearm might also be a factor in the rate of decolorization.

Several procedures were used to determine the effect of circulatory changes and anoxemia on the intradermal test. Arterial occlusion was produced by a blood pressure cuff for 5 to 15 minutes in one group of experiments. In another, pressure was applied to an area of the forearm for 5 minutes by a strip of X-ray film to which 2,000 gm of weight was attached. Both procedures resulted in a more rapid disappearance of the dye than in control tests. In the second experiment, the speed of decolorization may have been due in part to pressure which forced the dye into the deeper lymphatics rather than to ischemia of the underlying tissues. Changing the room temperature from 26°C to 18°C increased the time required for disappearance of the dye in one individual.

At times when a series of intradermal tests was performed in the same individual, one wheal would fade suddenly, while the remainder would disappear slowly over a long period of time. This phenomenon may be due to a sudden rupture of some of the tissue spaces.

Administration of 1,000 mg of vitamin C intravenously caused a rise in the ascorbic acid in the plasma from 0.15 to 1.40 mg/100 cc to a level of 6.0 to 8.0 mg/100 cc. During this period the decolorization time of the dye was reduced to about one-half of the original figure. Changes of this magnitude in the amount of vitamin C in the blood and tissues do not occur under ordinary circumstances.

Conclusion. In 100 tests there was no correlation between the amount of ascorbic acid in the blood during fasting and the time of decolorization of an intradermal injection of 2-6-dichlorophenol-indophenol. This skin test is not a satisfactory method for the determination of the state of vitamin C nutrition.

Electrophoresis of Epinephrine into the Skin. Application to the Treatment of Asthma.

HAROLD A. ABRAMSON.

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It has been known for many years that epinephrine may be transported by electrophoresis into the skin where it produces its typical blanching in the intact dermis. As far as the writer is aware, this phenomenon has not been applied to the electrical transport of comparatively large quantities of epinephrine into the skin to establish locally in the skin reservoirs of epinephrine for the relief of allergic states. A method of administering epinephrine by electrophoresis to form these reservoirs has now been developed. This technic and its application to the relief of severe asthma form the subject of this paper.

Preparation of the solution: With the current densities needed, solutions of epinephrine hydrochloride were too acid to employ. A solution of epinephrine dihydrogen phosphate between pH 3 and 4 was tentatively adopted. This solution is prepared by simply adding equivalent quantities of epinephrine base and phosphoric acid so that the final concentration of epinephrine itself is 1% (with suitable adjustment of pH).

Quantity transported: This solution contains mainly the ionic species:



Although the electric mobility of H^+ is much greater than either of the other 2 ions, its concentration is comparatively small even at pH 3.0. In the routine method now employed a single treatment lasts 30 minutes (occasionally more) with the current at 0.005 amp. Since 1.0 ampere is one coulomb per sec., approximately 9 coulombs are transported. Assuming that Faraday's laws hold here and that the electric mobility of the epinephrine ion is equal to that of $[\text{H}_2\text{PO}_4]^-$, it can be readily shown that the epinephrine ion accounts for the passage of approximately 2 coulombs; therefore, more than 2×10^{-5} moles of epinephrine are electrically transported into the skin. Since the molecular weight of epinephrine is near 183, about 4 mg of epinephrine are probably deposited in the skin by the method outlined in a single treatment.

Method of application: The epinephrine solution is applied on

cotton or canton flannel. The area of application (arm or leg) is 30 cm². Each area is treated 10 to 15 minutes, 2 to 3 areas being used successively in a single treatment. The blanching usually lasts more than 5 hours without injury to the skin. The rate of absorption from the blanched skin is retarded by the blanching itself. The epinephrine in the blanched areas is apparently pharmacologically active over this period and the epinephrine is slowly released from its depots in the skin. It is our view at present that the absorption rate is between 0.5 to 1.0 mg per hour.

Systemic reactions: The reactions (palpitation, tremor, etc.) observed have (with one exception) been less than those obtained with subcutaneous injection of 5 minims of the 1:1,000 solution. The effect of the electrophoretic administration on the blood pressure has been studied with Dr. M. A. Greene on a control normal group. There was frequently a slight drop (during the first 20 minutes) in the blood pressure which subsequently returned to its normal initial level. These data will be reported separately.

Results in asthma: Only hospitalized patients with severe asthma (in status asthmaticus or bordering thereon) were treated. The usual sedatives were administered, although the typical effects of administering epinephrine to asthmatics were encountered without sedation.

Although the number of patients (10) in this series is too small to anticipate the potentialities of this procedure, the therapeutic results appearing in Table I leave no doubt that the general pharmacological effects of epinephrine in asthma are obtained. In only one of the 10 cases summarized were the asthmatic paroxysms not relieved at least as much as by a therapeutic dose of an aqueous injection of epinephrine. Indeed, in several cases it seemed likely that the epinephrine depots formed in the skin by electrophoresis led to a more prolonged action. This is in keeping with the local effects obtained in the skin discussed in the foregoing. That is, the blanching lasts more than 5 hours. Furthermore, larger doses are administered in this way although the rate of absorption is curtailed. There may be some justification in comparing this method with an intravenous drip of epinephrine solution.

A case not listed in the table is described by Fig. 1. Note in Fig. 1, which is an illustration of treatment in a very severe case, that on the 2nd, 3rd, and 4th days the patient required very few injections of epinephrine subcutaneously. During this time epinephrine was administered by electrophoresis. Iontophoresis was discontinued on the 5th day when the patient's condition became worse. On the 6th

TABLE I.

Case No.	Sex	Age	No. of treatments administered	Clinical diagnosis	Pharmacologic effects of epinephrine on asthma
1	F	30	5	Constitutional Hypersensitiveness; Infectious Asthma	Obtained
2	F	55	1	Infectious Asthma	"
3	F	21	1	Infectious Asthma	"
4	F	60	4	Essential Hypertension; Congestive Heart Failure; Infectious Asthma	"
5	M	64	1	Atherosclerotic valvular disease; old coronary thrombosis; Acute coronary thrombosis; Infectious Asthma	Obtained partially
6	F	58	9	Infectious Asthma	Obtained
6a			26		
(Readmitted)					
7	F	45	2	Infectious Asthma	"
			(within 2 hr)		
8	M	24	6	Infectious Asthma; Psychoneurosis	Not obtained
9	F	38	8	Infectious Asthma; Lung Cyst	Obtained
10	F	58	3	Infectious Asthma; Myocardial Insufficiency	"

day epinephrine was administered by iontophoresis with only partial effect. On the 8th day and 9th day epinephrine in oil showed some slight effect. In this particular instance it seems evident that the pharmacologic effect of epinephrine was very well evident by the electrophoretic method. It should be noted that the care of the patient was not under the jurisdiction of the individual giving the epinephrine by electrophoresis. The nurse in charge of the patient administered epinephrine whenever the patient's condition warranted it. If at any time on the 2nd, 3rd, or 4th day conditions had required epinephrine, the nurse in charge would have given it, irrespective of the fact that epinephrine had been administered by ionto-

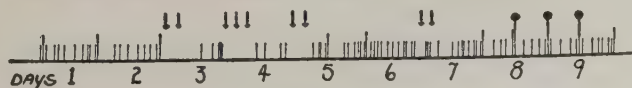


FIG. 1.

Diagram illustrating treatment of Mrs. B., a patient in status asthmaticus, by four different methods of administration of anti-asthmatics. The short vertical bars represent injection of 5 minims of 1:1000 epinephrine hydrochloride subcutaneously. The long vertical bars represent aminophyllin intravenously. The arrows pointing downward are treatments of epinephrine phosphate by electrophoresis. The longest vertical bars (8th and 9th day) surmounted by a closed circle are injections of epinephrine in oil.

phoresis. This, indeed, happened on the 6th day where the patient's condition was much worse and she was not relieved at all by injection of epinephrine and not as much as usual by the electrophoretic technic.

It is the impression of the writer that with further development and analysis of the technic here disclosed, more prolonged and effective action may be obtained than that observed with hypodermic administration.

Mrs. Henriette Gettner has been kind enough to render her able assistance. Schieffelin & Company have kindly supplied the epinephrine base.

10682

Dose-Response Relationship of Androsterone by Direct Application to the Capon's Comb.*

SAM R. HALL AND L. P. DRYDEN. (Introduced by M. H. Friedman.)

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Washington, D. C.*

Fussgänger¹ first discovered that the capon comb is very sensitive to the direct application of dissolved androgen. McCullagh and Osborn² use this method for the determination of androgens in the blood of men. Butz and Hall have taken advantage of the sensitivity of this method in a recent study³ wherein they showed that more of the androgen in bull's urine was found in the "cholestenone" than in the "cholestanone" fraction when the Anchel-Schoenheimer fractionation procedure was applied. None of the above authors has described fully the methods or the dose-response relationship for androsterone.

In an attempt to standardize the conditions in the use of this method and to understand the factors that influence the response, more than nine hundred individual tests have been made over a period of a year and a half. We have reported elsewhere⁴ that season, previous use,

* This research was supported by an appropriation from Bankhead-Jones funds (Bankhead-Jones Act of June 29, 1935).

¹ Fussgänger, R., *Mediz. Chem. Abhandl. Chem. Forschungstätten der I. G. Farbenin*, 1933, p. 213.

² McCullagh, D. R., and Osborn, W. O., *J. Biol. Chem.*, 1938, **126**, 299.

³ Butz, L. W., and Hall, S. R., *J. Biol. Chem.*, 1938, **126**, 265.

⁴ Hall, S. R., and Hunt, John D., *Proc. Am. Physiol. Soc.*, 1939, in press.

age after 6 months, and initial comb size were not detectable factors in the response. Continuous artificial light tried only in the fall of the year caused lessened response.⁵

The capons used in the experiment here reported were all of one strain, single comb, White Leghorn, all hatched at the same time and caponized at between 28-32 days of age. They were housed together and were on the same diet. The androsterone† was dissolved in tricaproin and so made up that the desired amount was contained in $\frac{1}{8}$ cc.‡ A 1 cc tuberculin syringe was used. When filled, it is, of course, sufficient for 8 applications and since the administration is repeated for 5 days on birds picked from the group at random, it is felt that the error in measuring $\frac{1}{8}$ cc in the syringe graduated in 32nds is within the limits of the accuracy claimed for this method.

Following Gallagher and Koch,⁶ length plus height was used as an index of comb size. Measurements were made with a transparent millimeter ruler. All but one barb had previously been removed from the combs by cautery and the backs of the combs cut smooth so as to eliminate any doubt regarding the points for measurement.

As a further check on our measurements the length and height of the comb on 20 unstimulated birds were determined twice on the same day with a mean difference of .1 mm between the 2 readings. Moreover as a further precaution in order to determine if comb manipulation and the tricaproin might not cause slight size increases in the comb, 13 birds were treated with the oil daily and the size changes noted on the sixth day. There was an average decrease of .5 mm with a S. E. of $\pm .3$; the decrease is most likely due to their continued regression following a use 3 weeks previously. It is probable that an average increase of 1 mm in 12 birds is significant.

"Slips"§ occasionally occur in our flocks. They appear most often at about the age of 4 to 5 months but have often been seen past a year and a half old. We can confirm Hooker and Cunningham⁷ in their conclusion that at least in these older birds this condition arises spontaneously and is not the result of incomplete castration. They are easily recognized and after they appear are, of course, not used.

⁵ Hall, S. R., and Dryden, L. P., *Proc. Am. Physiol. Soc.*, 1939, in press.

† Supplied by the Ciba Company through the courtesy of C. C. Haskell.

‡ We have evidence that for a given dose the volume of the vehicle is an important factor in the response and must be controlled.

⁶ Gallagher, T. F., and Koch, F. C., *J. Pharm. Exp. Therap.*, 1935, **55**, 97.

§ A term long used by poultrymen to designate unsuccessfully caponized birds. To avoid introducing a new word, we are using the term here to include also those cases in which complete gonadectomy seems to be followed by the production of new testicular tissue.

⁷ Hooker, C. W., and Cunningham, Bert., *Anat. Rec.*, 1938, **72**, 371.

TABLE I.

Date	No. of birds	Androsterone in gamma	Mean increase, mm	Stand. Dev.	Standard error
Oct. 31	6	.125	1.33	± 1.21	± 0.49
" 31	11	.250	3.73	± 1.21	± 0.37
" 31	11	.500	4.67	± 1.83	± 0.53
" 3	16	.500	4.69	± 1.62	± 0.41
" 3	16	1.000	6.25	± 2.21	± 0.55
" 3	16	2.000	8.50	± 2.22	± 0.56
" 3	16	4.000	11.13	± 2.42	± 0.60
" 3 (Injection)	15	100.000 ($\frac{1}{2}$ cc)	4.80	± 2.15	± 0.55

We, however, see no justification for discarding data obtained from them before they "slip" as did Gallagher and Koch.⁶ In reviewing our data on individual capons, we find that, as a rule, those which "slip" past 10 months or more of age have not been above the average in sensitivity previous to the time the comb and wattles become brilliantly red and fail, as they do, to regress following stimulation.

Table I gives the data for increasing doses of androsterone. The hormone was administered once a day for 5 days and the comb size increase determined on the sixth day.

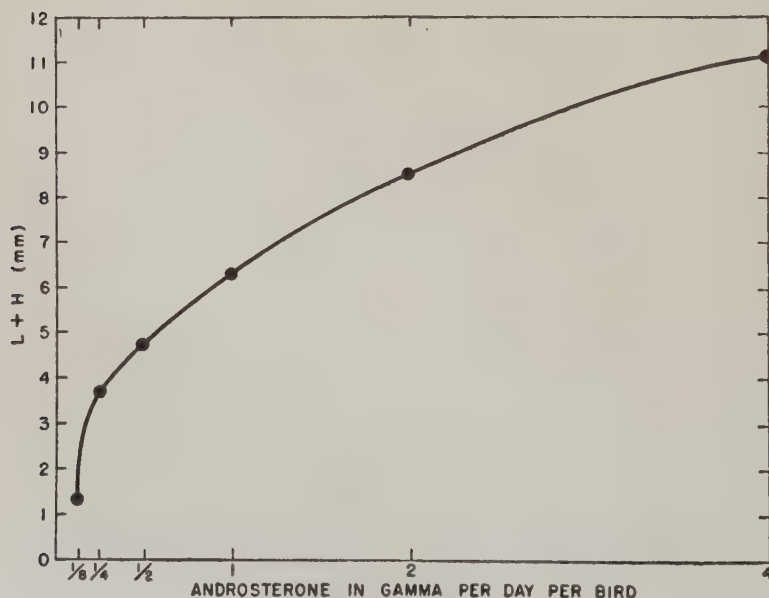


FIG. 1.

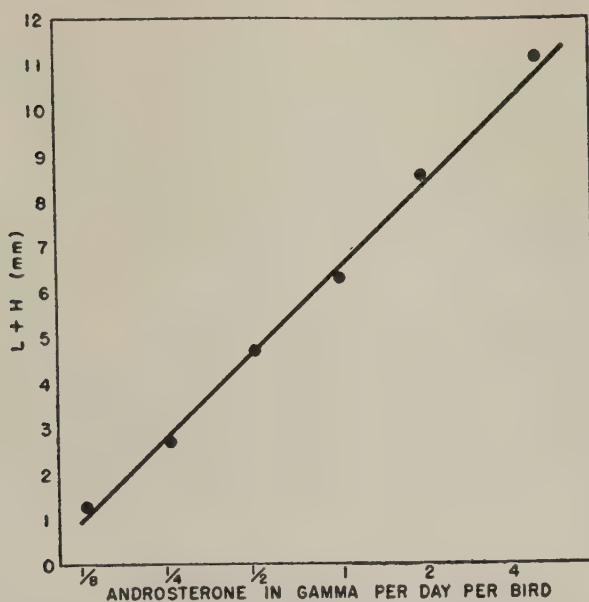


FIG. 2.

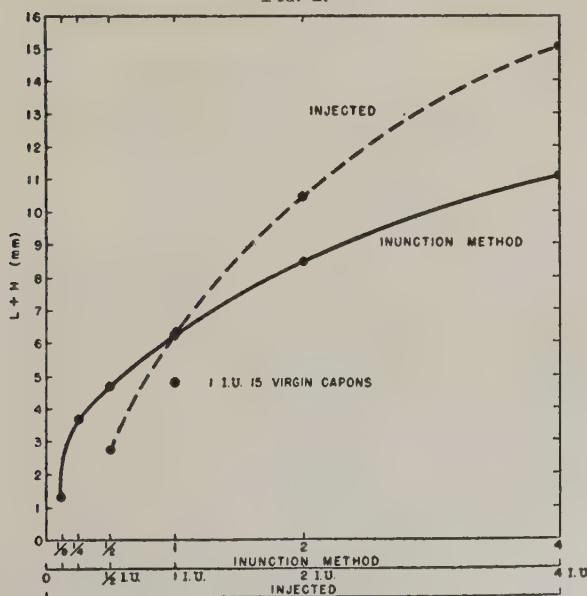


FIG. 3.

Fig. 1 gives the dose-response curve.¶ Sixteen capons 6-months-

¶ This curve is for androsterone in tricaproin. In work to be reported with L. W. Butz, we find the dilution factor to be important in the assay of crude

old that had not been used before were employed for all the points except .125 and .25 gamma. These were obtained 3 weeks later on 6 and 11 capons respectively. These 2 groups were so made up from the birds previously used only once that they should be equal to an average of the entire group. To find out if we were safe in combining data from "fresh" capons with those from birds previously treated, 11 of the capons selected as above were run in the second experiment on .5 gamma; these 11 gave a response identical with the first. (See Table).

From Fig. 2 it is seen that the curve is logarithmic from .125 to 4 gamma. The best fit is from .25 to 2 gamma.

Fig. 3 compares the curve in Fig. 1 with a curve obtained by injection. The latter curve is obtained from Greenwood, *et al.*⁸ For comparison, the average response obtained from the injection of 100 gamma androsterone into 15 of our "virgin" capons is included.

10683

Chemical Composition and Vitamin Content of Royal Jelly.*

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In the colony of the honeybee (*Apis mellifera* L.) there are two castes of females, the queen, whose function is limited to reproduction, and the workers, who carry on all the other activities of the colony. The physiological process by which one female larva develops into a worker and another becomes a queen is assumed to be determined by its diet. For the first 2 days after hatching all female larvae apparently receive the same diet and the physiology of their development is similar. This diet is royal jelly, a secretion of the pharyngeal glands of the workers, and it is fed to the larvae at fre-

extracts of bull and ram urine and presumably of extracts of other materials or fractions containing relatively large amounts of extraneous material. In one extract of ram urine, we were consistently unable to promote comb growth above 4.5 mm regardless of the concentration. For such materials we propose as an end point the maximum dilution that will cause unmistakable comb growth (1 to 2 mm).

⁸ Greenwood, Blyth, and Callow, *Biochem. J.*, 1935, **29**, 1400.

* A contribution from the Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture, in cooperation with the Louisiana State University, and the Bureau of Chemistry and Soils, U. S. Department of Agriculture.

quent intervals by the nurse bees. After the second day the diet of the larvae that are to become workers is altered, but the queen caste continues to receive nourishment from royal jelly.

Not only is anatomical differentiation brought about by this change in the worker's diet, but the rate of development is retarded. The imago of the worker emerges after 18 days, whereas the queen is mature in 13 days. The average weight of a female larva at the time of hatching is about 0.1 mg, and in 7 days the queen has increased her weight approximately 2,500 times and the worker about 1,400 times.

Since royal jelly is believed to be responsible for this remarkable development of the queen, a knowledge of its chemical composition and nutritional properties is important. Little work seems to have been done on this problem, however. Von Planta¹ made a chemical study of royal jelly in 1888, but apparently no one else reported any work along this line until 1922, when Aeppler² analyzed royal jelly. Later Elser³ determined the chemical composition of royal jelly taken from queen cells of various ages. Within the last few years several investigators have studied the vitamin E content of royal jelly in attempts to account for the extraordinary fecundity of queen bees on this basis. The experiments reported in this paper represent a part of a study of the physiology of reproduction of the honeybee.

Method of obtaining royal jelly. To provide sufficient quantities of royal jelly for chemical analysis and biological assay, the following method was used: A queenless and broodless colony was prepared with 3 to 5 pounds of bees and an ample supply of pollen and honey. Approximately 6 hours later 60 newly hatched female larvae were placed in small wax cell cups and given to the prepared colony. Twenty-four hours later, after being fed and cared for by the nurse bees, the cells containing the larvae were removed and distributed in groups of 20 to other colonies for finishing. After 48 hours the larvae were discarded, and the royal jelly was removed from the wax cells and stored at 0°C awaiting analysis.

Chemical analysis. The moisture was determined at 60°C in a vacuum oven. The Kjeldahl method was used for the estimation of total nitrogen. The quantity of nitrogen multiplied by the conventional factor 6.25 gave the value for protein. The total lipid was determined by the Kumagawa and Suto procedure as used by Slifer.⁴

¹ Planta, A. von, *Z. f. physiol. Chem.*, 1888, **12**, 327.

² Aeppler, C. W., *Gleanings in Bee Culture*, 1922, **50**, 151.

³ Elser, E., *Markische Bienen-Ztg.*, 1929, **19**, 232.

⁴ Slifer, E. H., *Physiol. Zool.*, 1930, **3**, 503.

The total reducing substance, calculated as glucose, was determined by a method suggested by Dr. Michael Somogyi, Jewish Hospital, St. Louis, Mo. To each gram of royal jelly in a 50 cc centrifuge tube 10 cc of 1.2N sulfuric acid was added. The tube was closed with a rubber stopper in which was inserted a 2-foot glass tube serving as a reflux condenser. The tube and its contents were heated for 3 hours in a boiling-water bath. When the digest was cool, it was neutralized with a saturated solution of barium hydroxide, phenolphthalein being used as an indicator. The solution was diluted to 100 cc and filtered. The total reducing substance in the filtrate was determined by the dinitrosalicylic acid method of Sumner.⁵ The writers believe that the total reducing substance is an index of the carbohydrate content of the royal jelly, because after fermentation with yeast the filtrates produced little, if any, color with the sugar reagent.⁶ Determinations of total reducing substance were made on protein-free filtrates of royal jelly and the values were in agreement with those obtained after acid hydrolysis.

The ash determinations were made in an electric muffle furnace at 600°C. The undetermined substances were calculated by difference.

The results of the chemical analysis of 8 samples taken over a period of 6 months are presented in Table I. These data demonstrate that royal jelly taken from queen cells containing larvae between 3 and 4 days old is of remarkably constant chemical composition, and are in agreement with results obtained by Elser.³

Biological value of royal-jelly protein. The results presented in Table I demonstrate that royal jelly provides an abundance of tissue-building material in the form of protein, as well as energy in its carbohydrate and total lipid. The biological value of royal-jelly pro-

TABLE I.
Chemical Composition of Royal Jelly.

Sample	Moisture %	Dry matter %	Protein %	Total lipid %	Total reducing substance %	Ash %	Unde- termined %
1	66.50	33.50	13.38	5.03	11.16	.88	3.05
2	66.38	33.62	12.38	5.92	12.99	.82	1.51
3	65.86	34.14	12.66	5.27	12.00	.81	3.40
4	65.55	34.45	12.16	5.51	11.61	.81	4.36
5	67.42	32.58	11.97	5.26	12.10	.82	2.43
6	66.44	33.56	11.75	5.51	12.70	.76	2.84
7	64.63	35.37	11.78	5.83	14.06	.82	2.88
8	65.60	34.40	12.63	5.38	13.27	.86	2.26
Av.	66.05	33.95	12.34	5.46	12.49	.82	2.84

⁵ Sumner, J. B., *J. Biol. Chem.*, 1925, **65**, 393.

⁶ Somogyi, M., *J. Biol. Chem.*, 1928, **78**, 117.

tein was determined by measuring the extent to which the protein is utilized by the standard white rat, employing the nitrogen balance method of Mitchell.⁷ Four white rats were fed for 3 experimental periods of 10 days each. During the first and third periods the rats received a nitrogen-free diet, and during the second period a diet containing royal jelly was fed. Thiamin, riboflavin, and vitamin B₆ were supplied to all experimental animals by feeding daily 1 cc of 1:1 dilution of Vitab Type II concentrate.[†] Feces and urine were collected during the last 7 days of each period. The average endogenous and metabolic nitrogen values were determined during the periods in which the nitrogen-free diet was fed. The diets are given in Table II.

TABLE II.
Diets Used in Studying the Biological Value of Royal-Jelly Protein.

Ingredient	Nitrogen-free diet %	Royal-jelly diet %
Butter	15	15.00
Lard	12	8.81
Cod-liver oil	2	2.00
Salt mixture	4	3.41
Starch	67	4.18
Royal-jelly-starch mixture	0	66.60

For the royal-jelly diet fresh royal jelly was mixed with starch and dried at a low temperature in a vacuum oven, and the dried mixture was thoroughly pulverized before the other constituents were added. The royal jelly was incorporated in such amount as to supply a protein level of 8%.

The nitrogen balance data are presented in Table III.

The average digestion coefficient was 81%, and the average biological value of royal-jelly protein was 75%. Mitchell⁸ has reported biological values for food proteins ranging from 38% for cooked navy beans to 94% for whole egg. Royal-jelly protein compares favorably with Mitchell's values for beef protein, which range from 69 to 77%.

The nutritional requirements for the growth of the honeybee and the white rat may be quite different, but it is of interest that the protein of royal jelly is adequate for the rat as shown by the data presented in Table III.

Vitamin studies with royal jelly. In addition to protein, carbo-

⁷ Mitchell, H. H., *J. Biol. Chem.*, 1924, **58**, 873.

[†] The vitamin concentrate was kindly supplied by Vitab Corp., Emeryville, Calif.

⁸ Mitchell, H. H., *J. Home Econ.*, 1927, **19**, 122.

TABLE III.
Nitrogen Balance of White Rats on a Royal-Jelly Diet for 10 Days Preceded and Succeeded by 10-Day Periods on a Nitrogen-Free Diet.

Rat	Initial wt, g	Final wt, g	Avg daily food intake, g	Avg daily N intake, mg	Avg daily fecal N, mg	Est. daily meta-bolic N, mg	Avg daily food N in the feces, mg	Avg daily absorbed N, mg	Avg daily urinary N, mg	Est. daily endogenous N,† mg	Avg daily food N in urine, mg	Avg daily food N utilized, mg	Digestion coeff-icent,‡ %	Biological value,§ %
Nitrogen-Free Diet.														
1	100.5	95.0	7.25		8.35	1.15			22.88	23.41				
2	123.0	119.0	5.84		11.10	1.90			27.76	22.94				
3	130.0	126.0	6.95		14.25	2.05			34.16	26.69				
8	105.0	98.0	4.73		12.65	2.67			37.76	37.20				
Royal-Jelly Diet.														
1	97.5	107.5	6.25	83.13	14.60	7.69	6.91	76.22	43.35	20.17	23.18	53.04	82	70
2	139.0	142.0	5.18	67.86	12.10	8.91	3.19	64.67	44.00	28.10	15.90	48.77	82	75
3	164.0	174.0	7.74	101.39	20.15	16.72	3.43	97.96	64.80	38.18	26.62	71.34	80	73
8	133.0	128.5	4.48	58.69	11.10	8.65	2.45	56.24	45.20	35.03	10.17	46.07	81	82
Nitrogen-Free Diet.														
1	144.0	137.0	3.64		4.75	1.30			22.40	15.94				
2	135.0	128.5	4.30		6.60	1.53			22.48	17.06				
3	162.0	148.0	3.45		7.80	2.26			28.64	18.48				
8	123.5	116.0	4.22		5.00	1.18			19.60	16.37				

* Calculated on the basis of 1 g of food.

† First and last groups of figures calculated on the basis of 100 g of live weight.

‡ (Food N — feces N)

× 100.

Food N

§ N intake — (fecal N — metabolic N) — (urinary N — endogenous N) × 100.

N intake — (fecal N — metabolic N)

hydrate, fat, and inorganic elements, certain vitamins are necessary for the development of an animal. Judging from what is known of the nutritional requirements of insects, one can conclude that insects require at least growth factors belonging to the vitamin-B group.

Royal jelly was assayed for its vitamin B₁ content by using the quantitative response of the white rat, since no reliable chemical means is available for the test. A modification of the Smith rat-curative procedure⁹ was used. Polyneuritis was produced in rats, and a single dose of the jelly was then administered to the animals. The response indicated that the jelly contained approximately as much vitamin B₁ as is present in whole wheat, or between 1 and 1½ international units per gram.

The vitamin A content of royal jelly was studied because there is some evidence that insects require this factor, which is essential to growth in young animals and to normal nutrition in adults.

Some preliminary tests showed that rats consumed royal jelly with avidity, and also that as much as 3 g fed daily for 3 days produced no demonstrable response in vitamin-A-deficient animals. The procedure given in the United States Pharmacopoeia XI for assaying cod liver oil was then followed, except that the number of animals was limited to 2 in each group and the jelly was fed at a level of 2 g daily. A litter of 6 animals was divided into 3 pairs. One pair received no vitamin A during the assay period and served as negative controls, another pair received daily 2 g of royal jelly, and the third pair received daily U.S.P. reference cod liver oil providing 2 units of vitamin A. The animals receiving the royal jelly failed as rapidly as did the negative controls, but the animals receiving the reference cod liver oil made a satisfactory response. It appears safe to conclude, therefore, that this sample of royal jelly was devoid of any demonstrable amount of vitamin A.

Hill and Burdett¹⁰ claimed that vitamin E was present in royal jelly and was responsible for the fertility of the queen bee. Vitamin E is a requirement for normal reproduction by certain mammals. In the female rat lacking vitamin E, pregnancy proceeds normally until a late stage, at which time the young die and are resorbed by the maternal organism. When sufficient vitamin E is provided pregnancy and the rearing of the young follow the normal course. Sterility in the female due to lack of vitamin E is curable with a small amount of this vitamin, but in the male it is incurable.

⁹ Kline, O. L., Tolle, C. D., and Nelson, E. M., *J. A. O. A. C.*, 1938, **21**, 305.

¹⁰ Hill, L., and Burdett, E. F., *Nature*, 1932, **130**, 540.

Because of the unsatisfactory nature of the rat assays conducted by Hill and Burdett, the problem was investigated more extensively by Mason and Melampy,¹¹ who found that when test animals were fed royal jelly in graded amounts, from 50 to 1,000 mg daily, in no case was there sufficient vitamin E to permit the completion of gestation in rats deficient in this vitamin. The results indicate that fertility of the queen caste of bees is not dependent on vitamin E as obtained in royal jelly. Schoorl,¹² Evans, Emerson, and Eckert,¹³ and Haydak and Palmer,¹⁴ confirmed these results.

Royal jelly was analyzed for vitamin C (ascorbic acid) according to the method of Bessey and King.¹⁵ Little, if any, of this vitamin was found to be present.

Summary. The composition and vitamin content of royal jelly, the substance responsible for the differentiation of the two castes of the female honeybee (*Apis mellifera* L.), has been determined by standard chemical methods and biological assays. Royal jelly has the following chemical composition: moisture, 66.05%; protein, 12.34%; total lipid, 5.46%; total reducing substance, 12.49%; ash, 0.82%; undetermined, 2.84%. Royal-jelly protein has an average digestion coefficient of 81% and a biological value of 75% as determined by the Mitchell method. Royal jelly proved to be a good source of vitamin B₁, containing from 1.0 to 1.5 international units per gram. It contained no demonstrable amount of vitamin A or vitamin C. It has been previously shown that royal jelly contains little, if any, vitamin E.

¹¹ Mason, K. E., and Melampy, R. M., *PROC. SOC. EXP. BIOL. AND MED.*, 1936, **35**, 459.

¹² Schoorl, P., *Z. Vitaminforsch.*, 1936, **5**, 246.

¹³ Evans, H. M., Emerson, G. A., and Eckert, J. E., *J. Econ. Ent.*, 1937, **30**, 642.

¹⁴ Haydak, M. H., and Palmer, L. S., *J. Econ. Ent.*, 1938, **31**, 576.

¹⁵ Bessey, O. A., and King, C. G., *J. Biol. Chem.*, 1933, **103**, 687.

Mammary Growth in Male Mice Receiving Androgens, Estrogens and Desoxycorticosterone Acetate.*

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The mammary ducts of male guinea pigs grew following the injection of $\Delta 5$ -transandrostenediol, testosterone propionate and 17-methyltestosterone.¹ The mammary tissue of immature male and female rats developed following injections of testosterone² and so did that of spayed virgin rats given androstenediol and androstenedione.³ A secretory response in the mammary gland of spayed pre-adolescent female Rhesus monkeys was produced by testosterone propionate, dehydro-androsterone, $\Delta 5$ -transandrostenediol and androsterone.⁴ (See Turner⁷ for review.) The injection of various estrogens and progesterone was followed by the growth of the mammary glands in male mice.^{5, 6}

The present experiment demonstrated the influence of various estrogenic and androgenic substances and desoxycorticosterone acetate§ on the mammary gland of young unoperated or castrated male mice weighing 16 to 25 g (Table I).

* These investigations were supported by the Belgian American Educational Foundation, the Anna Fuller Fund, and the Jane Coffin Childs Memorial Fund.

† Belgian American Educational Foundation Graduate Fellow.

‡ Rockefeller Fellow.

¹ Bottomley, A. C., and Folley, S. J., *Proc. Roy. Soc. B*, 1938, **126**, 224.

² Selye, H., McEuen, C. S., and Collip, J. B., *Proc. Soc. Exp. Biol. and Med.*, 1936, **34**, 201.

³ Nelson, W. O., and Gallagher, T. F., *Science*, 1936, **84**, 230.

⁴ Van Wagenen, G., and Folley, S. J., *Am. J. Anat.*, 1939, **73** (Suppl. 2), 54.

⁵ Gardner, W. U., Diddle, A. W., Allen, E., and Strong, L. C., *Anat. Rec.*, 1934, **60**, 457.

⁶ Gardner, W. U., Smith, G. M., Allen, E., and Strong, L. C., *Arch. Path.*, 1936, **21**, 265; Allen, E., Hisaw, F. L., and Gardner, W. U., Chap. VIII in *Sex and Internal Secretions*, Allen, Danforth, and Doisy, Editors, Williams and Wilkins Co., Baltimore, 1939.

⁷ Turner, C. W., Chap. XI in *Sex and Internal Secretions*, Allen, Danforth, and Doisy, Editors, Williams and Wilkins Co., Baltimore, 1939.

§ The androstenedione, testosterone, dehydro-androsterone, androsterone, $\Delta 5$ -trans-androstenediol, and desoxy-corticosterone acetate were generously supplied by Dr. E. Oppenheimer of Ciba Pharmaceutical Products, Inc.

The dibenzanthracene compound was supplied by Prof. J. W. Cook of the Royal Cancer Research Institute, London, the estradiol benzoate by the Schering Corporation through the courtesy of Dr. E. Schwenk, and the stilbestrol and triphenylethylene by Drs. G. A. Harrop and J. A. Morrel of the Squibb Institute of Medical Research and E. R. Squibb and Sons Biological Laboratories.

TABLE I.

	Size of glands	Total dose per mouse mg	Volume of oil per injection cc	Avg wt of mouse in every group g	No. of mice
Androstenedione	++++ or ++++	8	.1	23	5
Testosterone	++ to ++++	8	.1	17	10
	++ to ++++	4	.025	19	5
Dehydro-androsterone	++ to ++++	4	.05	22	10
Cis-androsterone	+	8	.1	24	5
Δ^5 -transandrostenediol	++ to 0	4	.1	26	10
Desoxy-corticosterone acetate	++++	4	.1	22	5
	+	2	.05	21	5
9:10 dihydroxy 9:10 di-n-propyl 9:10 dihydro- 1:2:5:6 dibenzanthracene	++++	1	.05	22	16
Idem, castrated mice	++++	—0.5	.025	17.5	5
Triphenylethylene	++	40	.05	15	7
	++ or ++++	10	.06	19	10
	++++	—5	.03	23	10
Estradiol benzoate	+	0.666	.05	19	5
	+	0.066	.05	20	5
	++	—0.016	.05	25	5
Stilbestrol	+	2	.05	21	5
	+++	0.2	.05	20	5
	++	—0.05	.05	23	5
Oil of sesame	0	—	.05	18	4
Not treated	0	—	—	21	5

The synthetic crystalline chemicals were dissolved in sesame oil and administered subcutaneously in 8 equal injections, one injection every other day. The mice were killed on the 16th day and all the mammary glands were studied (method described elsewhere⁵) for quantitative or qualitative differences of response. Usually 5 mice were used in each series.

Mammary glands of the untreated or oil-injected control mice consisted of one main duct with one or several branches. The total duct system extended for a length of one to 3 mm. The glands showing the least response are designated +. These glands equalled or slightly exceeded in size those of the oil-injected controls but showed an increased number of small, broader branching ducts usually terminating peripherally in nodular enlargements. These glands responded definitely but to a limited extent. Glands designated ++ were considerably increased in size and the duct system was increasingly complex. Growing buds were usually found at the distal ends of the peripheral ducts. The stages designated +++ and ++++ were developed more extensively. The spread glands in the latter stage exceeded 12 sq mm in area and showed a dense system of branching ducts. The presence of many terminal buds demonstrated that the growth was still in progress.

Administration of androsterone⁷ resulted in the least mammary growth, 8 mg giving a response of +. Similar mammary responses were obtained in mice which received 4 mg. $\Delta 5$ -transandrostenediol or large amounts of estradiol benzoate (0.666 mg to 0.066 mg) or stilbestrol (2 mg). Testosterone (4 and 8 mg), dehydroandrosterone (8 mg), large amounts of triphenylethylene (40 mg) and smaller amounts of estradiol benzoate (0.016 mg) or stilbestrol (0.2 to 0.05 mg) showed a ++ to +++ response. The greatest responses occurred in mice which had received androstenedione (8 mg), desoxy-corticosterone acetate (4.0 mg), 9:10 dihydroxy 9:10 di-n-propyl 9:10-dihydro-1:2:5:6 dibenzanthracene (1.0 or 0.5 mg) or triphenylethylene (10 or 5.0 mg).

The response of the glands of different mice in the same series was quite uniform. The individual mammary rudiments in some mice showed some variations in response, the larger ones being considered in the above experiments.

It is particularly interesting that the injections of 3 synthetic estrogenic chemicals differing rather strikingly from the naturally occurring estrogens; namely, triphenylethylene, stilbestrol and the dibenzanthracene compound are followed by mammary growth. The mammary growth in mice receiving desoxycorticosterone acetate

indicates again an overlap in physiological activity as well as the stimulating effect on the glands of animals receiving the various androgens.

The slighter mammary response in animals receiving the larger amounts of estradiol benzoate and also triphenylethylene and stilbestrol reaffirms the "stunting effect" on mammary tissues of large amounts of estrogens.⁶

10685

Effect of Sodium Dehydrocholate (Decholin) on Bile Salt, Chloride and Cholesterol of Bile in Dogs.

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Bile salts have been used for a number of years as choleretics. While it is generally accepted that the volume of bile after the administration of bile acids is increased,^{1, 2} few reports are available on the effect of these substances on the various constituents of the bile.^{7, 9} It is important to know whether or not following administration of certain bile salts, the patient excretes an increased amount of bile salt in the hepatic bile or whether the choleretic action is merely expressed by an increased water content. One of the most widely used choleretics is sodium dehydrocholate (Decholin). The following experiments were undertaken to determine what effect sodium dehydrocholate (Decholin) has on the 24-hour excretion of certain constituents of the bile.

Method. Two to 3 g sodium dehydrocholate (Decholin) were given daily for a period of 3 to 4 days to each of 5 dogs whose common duct had been doubly intubated by the method of Rous and McMaster,³ and whose cystic duct had been doubly ligated. Twenty-four-hour specimens of hepatic bile were collected before, during, and after the Decholin feeding. Bile was analyzed for chloride by the Wilson-Ball⁴ method, for cholate by the Gregory-Pascoe method

¹ Neubauer, E., *Klin. Woch.*, 1924, **3**, 883.

² Powelson, P., and Wakefield, E. G., *Ann. Int. Med.*, 1929, **3**, 572.

³ Rous, P., and McMaster, P., *J. Exp. Med.*, 1923, **37**, 11.

⁴ Wilson, D. W., and Ball, E. G., *J. Biol. Chem.*, 1928, **79**, 221.

⁷ Schmidt, C. R., Beazell, J. M., Atkinson, A. J., and Ivy, A. C., *Am. J. Digest. Dis.*, 1938, **5**, 613.

⁹ Doubilet, H., *Proc. Soc. Exp. Biol. and Med.*, 1937, **36**, 687.

TABLE I.
Changes in Composition of Bile After Administration of Sodium Dehydrocholate (Decholin) to Dogs.

Dog	Changes in Composition of Bile after Administration of Decholin						Grams Decholin ingested and period of ingestion						
	Volume cc			Chloride mg/24 hr			Cholate mg/24 hr			Cholesterol mg/24 hr			
	Before	During	After	Before	During	After	Before	During	After	Before	During	After	
247	121	195	92	329	617	237	3047	2051	2005	20	18	22	8 g 4 days
248	134	193	164	440*	581	439	2545	2704	3797	22	20	16*	9 g 3 "
346	75	100+	217	148	507	789	2030	3653	1389	13	8	8	6 g 3 "
			61			165			1233			9	
415	74	139	49	214	433	196	950*	1277	270	7	10	20	8 g 3 "
416	37	142	89	102	449	301	994	3834	1820	2	14	13	10 g 4 "
			66			231			2002			11	
Av.†	85	146	113	217	512	368	2114	2546	1721	12	14	15	

* One determination only.

† Average of all individual determinations.

as described by Reinhold and Wilson,⁵ and for cholesterol by a combined digitonin precipitation-colorimetric procedure.⁶

The period of control collection before Decholin administration was usually 4 days. The period of collection during Decholin feeding varied in the different dogs from 6 hours before the first Decholin feeding to 12 hours after the first Decholin feeding. The collection period after cessation of Decholin administration was from 2 to 4 days. In 2 animals bile was collected for a second "after" period, beginning about 8 to 10 days after Decholin feeding was terminated.

Table I gives mean figures for the volume and 24-hour excretion of cholate, chloride and cholesterol for each of the animals, and mean values for all 5 animals together.

The volume of bile increased after Decholin, and decreased in the "after" period. This increase is in agreement with that reported by all other workers, although it is greater than the 25% increase reported by Schmidt, Beazell, Atkinson and Ivy⁷ for a somewhat larger dosage of Decholin.

The concentration of chloride, as well as the volume of bile, rose with the result that the 24-hour output of chloride was enormously increased. The increase in chloride excretion is interesting in connection with a recent report by Rubin and Rapaport.⁸ They found that in animals with marked reduction in the plasma chloride concentration the mortality from anaphylactic shock was significantly reduced. Previous work in this laboratory had shown that the intravenous administration of sodium dehydrocholate for several days before a shocking dose of horse serum to sensitized dogs greatly reduced the symptoms of anaphylactic shock and the mortality consequent to a shocking dose of horse serum.

There was considerable variation in cholate excretion in different animals and in the same animal from day to day, but the mean values indicate that, after feeding Decholin, in 4 of 5 animals the cholate excretion increased, and in one it decreased. Mean values for the 5 animals together show an increase in cholate excretion of 20%. This is at variance with the results reported by Schmidt, *et al.*,⁷ who found Decholin to decrease the average 24-hour excretion of cholate in 3 dogs, and of Doubilet,⁹ who also reported cholic acid excretion to be decreased after feeding dehydrocholic acid, but found total bile acid to be increased, due to an increase in the desoxycholic acid fraction.

⁵ Reinhold, J. G., and Wilson, D. W., *J. Biol. Chem.*, 1932, **96**, 637.

⁶ Riegel, C., and Rose, H. J., *J. Biol. Chem.*, 1936, **113**, 117.

⁸ Rubin, M. I., and Rapaport, M., *Am. J. Med. Sc.*, 1939, **197**, 435.

The effect on cholesterol was extremely variable, there being in some cases an increase, in some a decrease.

Summary. Feeding sodium dehydrocholate to dogs with biliary fistulæ resulted in an increase in volume of bile excreted in a 24-hour period. Associated with the increase in bile volume there was an increase in the chloride excretion and in 4 of 5 dogs an increase in the cholate excretion. There was no significant change in 24-hour excretion of cholesterol.

10686

Experimental Catatonia in a Chimpanzee.*

H. DE JONG. (Introduced by J. F. Fulton.)

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Experimental catatonia, as defined by de Jong and Baruk,¹ consists of the following phenomena, which may be considered as an analogue of the syndrome of human catatonia: (a) After administration of an average dose of bulbocapnine or of other drugs having similar effect there occurs: (i) *Catalepsy, i. e.*, the tendency to retain for abnormally long periods of time postures imposed passively or assumed by the animals themselves. (ii) *Negativism, i. e.*, passive or active resistance against change of position, especially position in space. (iii) *Autonomic phenomena, i. e.*, polypnoea, salivation, etc. (b) After administration of a larger dose, hyperkinesia and abnormal postures are present. (c) After administration of a still larger dose epileptoid seizures may occur and lead ultimately to death.

Hitherto bulbocapnine had not been administered to an animal as closely related to human beings as a chimpanzee. Doctor John F. Fulton, to whom I am greatly indebted, gave me the opportunity to use 2 of his chimpanzees for this purpose. Two animals were injected (intramuscularly) with a dose of 10-15 mg per kg of body weight, 20 mg per kg being the average dose for the common macaque. The first animal, Chimpanzee "Ronald" (decorticated on one side one

* I am indebted to Doctor H. G. Barbour for providing me with the bulbocapnine used in this experiment.

¹ de Jong, H., and Baruk, H., *La catatonie expérimentale par la bulbocapnine*, Masson et Cie, Paris, 1930.

month previously), weighing 16.1 kg, after administration of 170 mg of bulbocapnine phosphate showed no symptoms other than a slight decrease of motor activity during a period of about 5 min; there was also bowing of the head and some salivation.

The second animal, however, showed symptoms of experimental catatonia completely identical with those known in other animals after administration of average doses of bulbocapnine. The following protocol gives the results of the experiment:

Expt. 1. Conspicuous cataleptic response of chimpanzee to bulbocapnine. Prompt recovery. ["Jiggs"]

The subject of the experiment was an unoperated male chimpanzee aged about 4 years and weighing 12.6 kg.

At 2:18, February 2, 1939, it received an injection (I.M.) of 140 mg bulbocapnine phosphate. Signs of action of the drug proceeded as follows:

2:25 p.m. Drowsiness.

2:28, p.m. The animal lay down and salivated profusely, and on being touched with a stick did not move away.

3:00 p.m. Marked negativism with strong resistance to change of position in space. Catalepsy was easily demonstrated in the following way: the animal was placed with the forelegs on one chair and with the hind legs on another. The chimpanzee remained in this position when the experimenter increased the distance between the 2 chairs, the animal became stretched out and offered no resistance to the manoeuvre. The state just described continued without essential change until 3:25 p.m.

3:25 p.m. The animal came out of the catatonic state, and when touched with a stick walked away.

3:30 p.m. Reinjection with 70 mg bulbocapnine phosphate.

3:33 p.m. Some decrease of motor initiative and bowing of head. Animal sat down in a remarkable posture, crossing the hind legs, and assumed a posture reminding one of a Buddha.

4:00 p.m. Coming out of the above described state, the chimpanzee walked around. Reinjection (I.M.) with 80 mg bulbocapnine phosphate with reappearance of catalepsy and negativism for a period of about one-half hour.

Summary. We have described the effect of intramuscular injection of 10-50 mg bulbocapnine phosphate per kg in chimpanzees. One of the animals showed symptoms of experimental catatonia identical with those known to follow the administration of an average dose of this drug in other animals.

10687 P

An Apparent Sex-Specificity in the Action of Progesterone on Adrenalectomized Cats.

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Stewart¹ first called attention to what he considered an unusual prolongation of the life-span of pregnant cats following adrenal removal. Later, Stewart and Rogoff,² working with dogs, reported similar findings and suggested the corpus luteum as the life-maintaining tissue in such animals. The writer³ was subsequently unable to substantiate these claims in a study of an extended series of pregnant and lactating cats. Recent reports of prolonged survival in adrenalectomized pseudopregnant dogs,^{4, 5} rats,^{6, 7} and ferrets,^{8, 9} together with the availability of progesterone in synthetic form, have led to a revival of interest in this phase of adrenal physiology.

Eight female and 2 male cats were adrenalectomized, and injections of progesterone ("Proluton"*) were begun at the first appearance of definite symptoms of cortico-adrenal insufficiency (anorexia, weakness, uncertain gait). The concentration of the blood glucose was followed throughout all experiments, and terminal determinations of the serum sodium, chloride, potassium and urea were made, as well as liver, muscle and cardiac glycogen.

The results obtained from the injection of progesterone into male animals indicated that the hormone served as an adequate replacement therapy in the absence of the adrenal cortex. Particularly notable was the restoration of normal carbohydrate levels following post-adrenalectomy depletion. The recovery of male cats from definite

¹ Stewart, H. A., Abstract published in 17th Internat. Con. Med. (London), Sec. III, 1913.

² Stewart, G. M., and Rogoff, J. M., *PROC. SOC. EXP. BIOL. AND MED.*, 1925, **22**, 394.

³ Corey, E. L., *Physiol. Zool.*, 1928, **1**, 147.

⁴ Rogoff, J. M., and Stewart, G. N., *Am. J. Physiol.*, 1928, **96**, 20.

⁵ Swingle, W. W., Parkins, W. M., Taylor, A. R., Hays, W. H., and Morrell, J. A., *Ibid.*, 1937, **119**, 675.

⁶ Emery, F. E., and Schwabe, E. L., *Endocrinol.*, 1936, **20**, 550.

⁷ Cavanaugh, C. J., and Gaunt, R., *PROC. SOC. EXP. BIOL. AND MED.*, 1937, **37**, 226.

⁸ Gaunt, R., *Cold Spring Harbor Symp. Quant. Biol.*, 1937, **5**, 395.

⁹ Gaunt, R., and Hays, H. W., *Am. J. Physiol.*, 1938, **124**, 767.

* "Proluton"—synthetic, crystalline progesterone, furnished through the generosity of the Schering Corporation, Bloomfield, N. J.

symptoms of cortical insufficiency was rapid, and similar to that seen in cats treated with cortico-adrenal extract. The blood chemistry at this time was found to be essentially normal. The injection of progesterone was stopped 21 days after operation when both animals were in excellent condition. They subsequently succumbed, with typical symptoms of adrenal insufficiency and careful examination failed to reveal any trace of adrenal tissue.

Non-pregnant female cats, on the other hand, showed no favorable reaction to progesterone injection. All succumbed, indeed, within the usual life-span of untreated animals and with typical symptoms of adrenal insufficiency, despite increased progesterone dosage. The blood and tissues showed the usual abnormal chemical values characteristic of untreated adrenalectomized animals.

Gaunt⁸ has shown that in the ferret the symptoms of adrenal insufficiency are accentuated by the injection of estrone. The possibility that the cats used in the present experiments, adrenalectomized in the spring, possessed a sufficiently high estrone content in their blood to vitiate the progesterone therapy should be considered as an explanation of the apparent sex-specificity noted herein.

One pregnant animal, restored from symptoms of adrenal insufficiency, survived 17 days during progesterone treatment. It then succumbed, however, with typical symptoms, although the dosage of progesterone was increased from 10 to 12 mg per day. The abnormally extended survival period of this animal may be accounted for on the basis of additional progesterone secretion from the corpora lutea present in its ovaries, as well as the absence of estrone. These probably favorable conditions, however, failed to maintain life indefinitely.

10688

Relation of Spermatogenesis to the Factor in the Testis Which Increases Tissue Permeability.

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The ability of aqueous extracts of the mammalian testis to increase the dermal spread of particulate matter has been demonstrated by

Duran-Reynals¹ and others.² Claude and Duran-Reynals³ showed that extracts of other organs in the normal adult mammal also possess this ability, although to a much lesser extent, indicating that possibly this spreading capacity is primarily a property of the testis. On the other hand, a number of actively growing mammalian tumors as well as placental tissue and mammalian embryos have been found to be high in spreading factor content.^{4, 5} A consideration of these facts has led Boyland and McClean⁵ to suggest the possibility that the spreading factor is a property of any rapidly proliferating tissue, of which the testis is but one example.

In the hope of throwing light on this interesting question we have determined the diffusing activity of extracts of testis in which spermatogenesis was inactive. In such an experiment one is, of course, employing testis tissue, but testis tissue in which the rate of cell proliferation is quite low or absent. For our purpose the immature testis and the cryptorchid testis were chosen.

The immature testes were obtained from 18 rabbits weighing about 800 g. These testes, which weighed between 170 and 900 mg were

TABLE I.
Spread of India Ink by Aqueous Extracts of the Testes of Immature and Mature Rabbits.

Rabbit No.	Immediate cm ²	1 hr cm ²	4 hr cm ²	24 hr cm ²
1:1 Dilution.				
1, 2, 3	3.22	9.35	15.30	18.51
4, 5, 6	3.21	6.39	8.99	12.96
7, 8, 9	3.06	7.13	9.34	13.59
10, 11, 12	3.02	6.04	8.24	11.68
Mean	3.11	6.81	8.73	13.18
Mean of controls	3.44	6.08	6.28	7.64
Mean of 20 adult testes, 1:1 dilution	*	19.27	31.39	27.68
1:5 Dilution.				
13	3.33	6.13	9.58	4.51
14	3.27	5.72	9.53	6.37
15	3.18	6.24	8.46	4.32
16	3.09	5.68	6.65	2.84
17	2.90	6.25	6.90	3.55
18	2.58	4.97	7.61	3.87
Mean	3.16	5.92	8.65	4.65
Mean of controls	3.01	5.70	6.93	3.57
Mean of 20 adult testes, 1:5 dilution	6.13	9.34	11.89	10.68

* Spread too fast to obtain reading.

¹ Duran-Reynals, F., *Compt. rend. Soc. biol.*, 1928, **99**, 6; *J. Exp. Med.*, 1929, **50**, 327.

² McClean, D., *J. Path. and Bact.*, 1930, **33**, 1045; 1931, **34**, 459.

³ Claude, A., and Duran-Reynals, F., *J. Exp. Med.*, 1934, **60**, 457.

⁴ Duran-Reynals, F., and Stewart, F. W., *Am. J. Cancer*, 1931, **15**, 2790.

⁵ Bayland, E., and McClean, D., *J. Path. and Bact.*, 1935, **41**, 553.

triturerated separately with an abrasive. Six testes were mixed with 5 times their weight of Locke's solution and the remainder were grouped into 4 lots and mixed with an equal weight of Locke's solution. After centrifugation the supernatant fluids were mixed with equal volumes of India ink in a 1:2 dilution with Locke's solution. The resulting mixtures were injected in 0.5 cc amounts intradermally in adult rabbits. As a control the same rabbits were also injected with India ink diluted 1:5 with Locke's solution. For comparison 20 adult rabbit testes were tested in 1:1 and 1:5 dilutions. Tracings were made of the spread of the ink in the injected rabbit and the area was determined with a planimeter.

The results are shown in Table I. The immature testes contained decidedly less of the spreading factor than did the adult testes; but it is interesting that the spread obtained from the immature testes was related inversely to the weight of the testes.

Although their small size precluded histological study of the immature testes, sections of testes of rabbits of similar size revealed that the tubules were immature. Some of the cells were undergoing mitotic division but no mature sperm were being formed.

In the study of the cryptorchid testis 14 adult white rats were used. Four of these animals were bilaterally cryptorchidized and 4 were unilaterally cryptorchidized by occlusion of the internal inguinal rings with black silk sutures after elevation of the testes into the abdomen and section of the gubernaculi. These animals were killed 45 days after the operation along with 6 normal rats. A section of each testis was made for histological study. The remainder of each testis was ground and extracted with an equal weight of Locke's solution. After centrifugation the supernatant fluid was mixed with equal parts of India ink in a 1:2 dilution, and the resultant mixture was injected in 0.5 cc volumes intradermally in rabbits. The area of the spread of the ink was ascertained as before by planimeter measurement of tracings of the area.

As shown in Table II the difference in the spread of the ink by extracts of the scrotal and the abdominal testis was quite marked, with the abdominal testis possessing approximately one-half of the spreading power of a scrotal testis. The spread by the abdominal testis of the unilateral cryptorchids was possibly significantly less than by the testis of the bilateral cryptorchids, while the scrotal testis of the unilateral cryptorchids had about the same capacity of this nature as the testis of the normal rats.

Histologically the cryptorchid testes showed the usual atrophy and hyalinization of the tubules without alteration of the intertubular elements. In our animals the atrophy was more marked in the uni-

TABLE II.
Spread of India Ink by Extracts of the Testes of Cryptorchidized and Normal Rats.

Rat No.	Immediate cm ²	1 hr cm ²	4 hr cm ²	24 hr. cm ²
Bilateral Cryptorchids.				
1	3.40	9.10	9.07	10.19
2	3.31	10.60	13.85	15.70
3	3.13	14.58	25.96	30.46
4	2.36	8.25	16.10	16.73
Mean	3.05	10.63	16.25	18.27
Unilateral Cryptorchids.				
Scrotal Testis.				
5	3.20	24.52	38.76	34.72
6	3.37	25.94	33.86	34.02
7	2.99	20.61	26.31	30.96
8	3.00	12.48	19.65	*
Mean	3.14	20.89	29.65	33.23
Unilateral Cryptorchids.				
Abdominal Testis.				
5	2.99	11.76	11.74	13.39
6	1.98	6.53	6.08	8.99
7	2.81	10.55	13.00	12.74
8	2.48	8.23	13.75	14.67
Mean	2.57	7.07	11.14	11.45
Normal Animals.				
9	2.81	17.39	20.48	20.48
10	6.62	30.79	48.87	57.40
11	6.58	19.97	30.00	36.69
12	4.42	22.53	18.74	20.12
13	4.26	25.84	34.32	38.76
14	4.25	20.73	19.06	17.92
Mean	4.82	22.88	28.58	31.89

* The spread was too diffuse to measure.

lateral cryptorchids than when both testes were abdominal. The degree of atrophy in these cases corresponds with the extent of diminution of the spreading factor. In certain instances among the bilateral cryptorchids there was a slight persistence of spermatogenesis. In all the testes the extent and amount of spermatogenesis corresponded quite closely with the amount of spread of the ink by their extracts.

These experiments show that for a testis to possess the spreading factor in large quantity the testis must be undergoing active spermatogenesis, and it is possible that complete spermatogenesis is essential. This fact was indicated by Hoffman and Duran-Reynals⁶ and McClean² who reported, without details, that in 4 experiments the cryptorchid testis contained less than normal testicular spreading capacity. They also reported that rabbit spermatozoa from the epididymis possessed the factor.

Thus it appears that the spreading factor is probably a characteristic of rapidly proliferating tissue and its high concentration in nor-

⁶ Hoffman, D. C., and Duran-Reynals, F., *J. Exp. Med.*, 1931, **53**, 387.

mal adult testis is the result of this fact. Whether this is the only factor involved, however, seems unlikely as one of us⁷ has shown elsewhere that India ink spreads over a large area in castrated male rabbits than in normal males.

Summary. A study of the amount of spreading factor present in the undescended testes of young rabbits and of the cryptorchidized testes of mature rats showed that this factor was greatly reduced. From this we concluded that the spreading factor was related to spermatogenesis.

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Influence of Diet on Intoxication with Phenol and Cyanide.

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The influence of diet on resistance to disease and to various intoxications has been recently reviewed.^{1, 2} The rôle of the vitamins has been extensively studied, but relatively little attention has been paid to the proportions of protein, carbohydrate and fat in the diet. From available reports it would appear that in some conditions one of these foodstuffs possesses an advantage, and in other conditions another. Many of these studies are of doubtful value since the vitamin factor was inadequately controlled. The present work was undertaken to compare the effects of diets high in protein, carbohydrate or fat on certain intoxications for which a definite detoxication mechanism is known. This report deals with 2 such poisons—phenol, which is detoxicated in part as an ethereal sulfate and in part in combination with glycuronic acid, and cyanide, which is in part detoxicated by conversion into thiocyanate.

Plan of Study. Young rats from a mixed albino and hooded Norwegian colony, weighing 60 to 70 g, were placed in separate cages and given diets varying in their content of protein, carbohydrate and fat. Littermates were divided equally in the various experimental groups. After an interval varying from 10 to 24 days on the test diet they were injected subcutaneously with a 5% aqueous solution of phenol or a 0.1% solution of NaCN freshly made. The mortality

⁷ Sprunt, D. H., and McDearman, S., in preparation.

¹ Clausen, *Physiol. Rev.*, 1934, **14**, 309.

² Robertson, *Medicine*, 1934, **13**, 123.

of these animals was then compared and contrasted with similar animals on a stock diet. The composition of the experimental diet is given in Table I. The stock diet had the following composition: yellow ground corn, 57.0; whole milk powder, 25.0; linseed oil meal, 12.0; crude casein, 3.7; alfalfa meal, 1.5; NaCl, 0.4, and CaCO_3 , 0.4. The mortality of 92 animals given phenol and 194 animals given cyanide is given in Tables II and III.

TABLE I.
Experimental Diets.

	High Protein	High Carbohydrate	High Fat
Casein	18.75	5.00	5.00
Dextrose	0.30	18.70	0.12
Olive oil	0.37	0.22	—
Lard	—	—	8.00
Halibut liver oil	0.30	0.30	0.30
Wheat germ oil	0.03	0.03	0.03
Brewer's yeast powder	1.25	1.25	1.25
Mineral mixture*	1.00	1.00	1.00

* This consisted of a slight modification of the Cox-Imboden salt mixture.³ Its composition was as follows: CaHPO_4 , 25 parts; NaCl, 18.4 parts; MgSO_4 (anhydrous), 6.86; KCl, 2.88; KHCO_3 , 44.4; Fe citrate, 2.21; $\text{CuSO}_4 \cdot 5 \text{H}_2\text{O}$ —0.24; $\text{MgSO}_4 \cdot 7 \text{H}_2\text{O}$ —0.03; KI, 0.015; NaF, 0.03.

TABLE II.
Mortality of Rats Given Phenol.

Exp. No.	I	II	III
Days on experimental diet	16	23	24
Dose of phenol, g/kg	0.5	0.6	0.6
Mortality (deaths/total rats)			
High protein diet (avg wt = 100 g)	1/9	3/10	2/11
High carbohydrate diet (avg wt = 131 g)	3/10	5/9	5/11
High fat diet (avg wt = 129 g)	4/9	0/8	5/11
Stock diet (avg wt = 108 g)			4/10

TABLE III.
Mortality of Rats Given Cyanide (7 mg NaCN per kg).

Exp. No.	IV	V	VI		Mortality	Avg wt
Days on exp. diet	10	10	14	Total	%	g
Mortality (deaths/total rats)						
High protein diet	7/8	7/10	8/8	22/26	85	80
High carbohydrate diet	8/10	8/10	7/8	23/28	82	105
High fat diet	4/10	6/8	5/8	15/27	56	109
Stock diet		8/11		8/11	73	105
Exp. No.	VII	VIII	IX		Mortality	Avg wt
Days on exp. diet	18	20	22	Total	%	g
Mortality (deaths/total rats)						
High protein diet	0/8	6/8	6/9	11/25	44	98
High carbohydrate diet	3/5	5/10	6/10	14/25	56	118
High fat diet	0/8	2/10	1/9	3/27	11	114
Stock diet	2/9	5/10	5/0	12/25	48	106

³ Cox and Imboden, *Proc. Soc. Exp. Biol. and Med.*, 1936, **34**, 443.

Results. In the animals poisoned with phenol no consistent difference was noted between the fat and carbohydrate groups. The animals receiving protein showed a somewhat lower mortality. It is to be noted, however, that the animals fed high protein had not gained as much weight as the other groups, and, since the dose was based on body weight, these animals therefore received a somewhat smaller dose of phenol than the animals in the other groups of the same age.

With cyanide, we have tabulated separately the animals who were on the experimental diet only 10 to 14 days. Even in this group, however, it is noticeable that the mortality of animals on a high fat diet is distinctly less than on the high carbohydrate, high protein or stock diet. When the experimental diet was continued for 18 to 22 days this difference becomes very striking—the mortality in the high fat group being only 11% as contrasted with 56% for the high carbohydrate group and 44% for the high protein group. We have no explanation to offer for the marked protection against cyanide poisoning afforded by a high fat diet.

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Influence of Diet on Resistance to Diphtherial Toxin

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In order to investigate the influence of diet on the resistance of rats to diphtherial toxin, groups of rats were placed on diets rich in protein, carbohydrate, and fat respectively. The diets and conditions of the experiment were identical with those described in the preceding paper.¹ After 17 to 21 days on the experimental diet the rats were given a subcutaneous injection of diphtherial toxin* (7 m.l.d. per gram of rat). Relatively large doses of toxin are required to kill these highly resistant animals. According to Cobbet² and Ramon, *et al.*,³ the rat requires from 1250 to 1750 times as much toxin per unit of animal weight as does the guinea pig. Preliminary experi-

¹ Meyer, A. R., *PROC. SOC. EXP. BIOL. AND MED.*, 1939, **41**, 402.

* The concentrated toxin used in these experiments was supplied by E. R. Squibb and Sons, through the courtesy of Dr. G. W. Bake.

² Cobbet (1899), quoted by Pettit, *Ann. de l'Inst. Pasteur*, 1919, **28**, 663.

³ Ramon, Nouredidine, and Erber, *Compt. Rendu Soc. de Biol.*, 1928, **99**, 562.

TABLE I.
Mortality of Rats Receiving Diphtherial Toxin* (7 m.l.d. per g).

Exp. No. Days on exp. diet	I 17	II 18	III 20	IV 21	Total	Mortality %
Mortality (deaths/total rats)						
High protein (avg wt = 83 g)	1/4	3/7	0/5	0/9	4/25	16
High carbohydrate (avg wt = 130 g)	4/4	5/6	3/5	5/10	17/25	68
High fat (avg wt = 124 g)	3/4	3/6	4/6	6/8	16/24	67
Stock (avg wt = 80 g)	3/4			2/4	5/8	63
Inanition 2-4 days (avg wt = 80 g)	1/5			1/6	2/11	18

ments with our rat colony indicated that the dose given above would kill 2 out of 3 rats in about 5 days. The mortality-data on the different diets are given in Table I. In fatal cases death occurred on an average about the fifth day in all groups of animals.

Results. An examination of Table I shows no difference in mortality between the high-fat and high-carbohydrate groups, but a very striking reduction of mortality in the case of the high-protein diet. One can not, however, conclude from this that protein is a superior food as far as increasing resistance to diphtherial toxin is concerned. Attention must be called to the fact that the high-protein animals had gained less weight than either the high-fat or the high-carbohydrate groups and that they consequently received a smaller absolute amount of toxin than either the high-fat or high-carbohydrate animals. Retardation in growth occurs inevitably when an animal's intake of protein is suddenly increased. The studies of Addis⁴ and others have shown that under these conditions hypertrophy of various organs, particularly the liver, takes place, and time must be allowed for this adjustment to occur before the animal can thrive and grow on the new diet. In order to find out whether inanition could be playing a part in inducing the increased resistance of the high-protein-fed animals, it was decided to study the effect of acute inanition *per se*. Two groups of animals on stock diet were fasted 2 to 4 days and were then given doses of toxin based on body-weight as described above. The results are shown at the foot of Table I. It appears that acute inanition will cause a marked increase in resistance to diphtherial toxin when doses are based on body-weight.

It is possible that inanition played some part in increasing the resistance of our high-protein animals, but one is not justified in attributing their increased resistance to this alone, for at the time the high-protein animals were injected with toxin they were no longer suffering from inanition but were eating and gaining well.

⁴ Addis, T., *J. Biol. Chem.*, 1936, **110**, 343.

TABLE II.
Mortality of Animals Receiving Diphtherial Toxin (900 m.l.d. per rat).

Exp. No.	V	
Days on experimental diet	21	Mortality %
Mortality (deaths/total rats)		
High fat (avg wt = 130 g)	5/8	63
High carbohydrate (avg wt = 132 g)	5/10	50
High protein (avg wt = 104 g)	2/8	25

The similarity of the results in the high-protein animals and the animals with acute inanition may have been due to the fact that the animals suffering from acute inanition were in reality living on a high-protein diet derived from their own body protein.

A further experiment was carried out to see if the increased resistance of the high-protein group—as contrasted with the high-carbohydrate and high-fat groups—would be evident if all animals were given the same absolute dose of toxin regardless of weight. The smaller protein-fed animals would then be receiving a larger per kilogram dose of toxin than the other groups. It was felt that if, under these circumstances, they still showed a higher resistance than the high-carbohydrate or high-fat groups, the conclusion would be justified that a high-protein diet really offered superior protection against the toxin. The results of this experiment are shown in Table II, in which animals in all groups received 900 m.l.d. of toxin.

It is apparent that even under these adverse conditions the high-protein-fed rats make the better showing.

Summary. 1. Rats fed on a diet rich in protein (casein) for 17 to 21 days showed a markedly higher resistance to diphtherial toxin than rats fed diets high in carbohydrate or fat. This could be demonstrated not only when the dose of toxin was based on the weight of the animals, but also when the dose was based on age. 2. Acute inanition caused an increased resistance to diphtherial toxin when the dose was based on the weight of the animal.

Cultivation of Vaccinia in Agar-Slant Tissue Cultures.

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The method most widely used for *in vitro* cultivation of vaccine virus is the modified Maitland technic with chick embryonic tissue suspended in Tyrode's solution. In this medium, multiplication of the virus occurs in the cells but not in the fluid in which they are suspended. It seems conceivable, therefore, that any medium which is capable of preserving metabolic activities of the cell may also provide conditions necessary for the growth of vaccine virus. Zinsser, *et al.*,^{1, 2} devised a solid medium, consisting of Tyrode's solution, horse or beef serum and agar, which the authors successfully employed for the cultivation of different Rickettsiae. Since these organisms are intracellular parasites and require for their growth the presence of viable cells, it is likely that the medium permitting their development may also be satisfactory for the cultivation of vaccine virus. The medium has already been successfully used in this laboratory by FitzPatrick for the cultivation of the Eastern strain of equine encephalitis. In the present paper, the successful cultivation of vaccine virus is described.

The virus used was a strain of vaccinia propagated for some time in chorio-allantoic membranes of developing hen's eggs. The material for initiation of cultures was prepared by removing an infected membrane and mincing it in a large test-tube with scissors. A small, arbitrary amount of this material was then inoculated into finely minced tissues of chick or mouse embryo and again thoroughly mixed. The inoculated tissue was deposited upon agar slants prepared as described by Zinsser and his coworkers. From 60 to 80 mg of the tissue by wet weight was placed upon each agar slant contained in test-tubes measuring 6x1 inches. Usually, the amount of tissue obtained by mincing one chick embryo of 8 to 9 days old or that of a mouse weighing 0.5-0.6 g was sufficient for inoculation of 6 or 8 agar slants. The tubes were tightly stoppered with rubber stoppers and kept at 37°C for 5 days. Subsequent serial transfers were made by inoculating freshly prepared chick or mouse embryonic tissues with the contents of one agar-slant culture. In this manner,

¹ Zinsser, H., Wei, H., and FitzPatrick, F., *Proc. Soc. Exp. Biol. and Med.*, 1938, **38**, 285.

² Zinsser, H., FitzPatrick, F., and Wei, H., *J. Exp. Med.*, 1939, **69**, 179.

the virus has been propagated for 16 generations in chick embryonic tissue and for 12 generations in mouse embryo. Determinations of potency were carried out every 3rd generation of both series. Since the initial cultures were inoculated with an arbitrary amount of an infected chorio-allantoic membrane, the determination of the titer of these cultures before incubation was also performed. In all instances, the material for titration was weighed under sterile precautions, ground, diluted to different concentrations, and 0.2 cc of each concentration injected into shaved rabbits' skins. It was found that the infected tissue used for the first cultures and titrated before incubation gave positive skin reactions in dilution 1:250. The culture grown in chick embryonic tissue reached in the 3rd generation the titer of 1:50,000. The 6th generation showed a titer of 1:100,000. This titer remained unchanged for the 9th and 12th generations. The 15th generation gave a titer of 1:1,000,000. The cultures propagated in mouse embryo increased to 1:100,000 in the 6th generation, but the titration of the 9th generation gave only 1:10,000. The 12th generation showed again the titer of 1:50,000. These fluctuations of titer of cultures propagated in mouse embryonic tissue were thought to be due to the inferior qualities of the mouse embryo as compared with those of chick. To prove this point, the 9th generation culture grown in chick embryo, showing a titer of 1:100,000, was transferred into mouse embryo tissue. Subsequent titrations of this new culture indicated immediate and considerable drops in potency of the original culture to 1:10,000 and 1:1,000 in the 1st and 3rd generations respectively. When, however, the latter culture of a low titer was transferred back into chick embryo, rapid rise of the titer to the original level of 1:100,000 was observed. Likewise, the 9th generation of the culture grown in mouse embryo tissue having a titer of 1:10,000 was transferred into a chick embryo tissue culture and an immediate rise of the titer to 1:10,000 was recorded.

It is difficult to explain why the cultures initiated with the infected chorio-allantoic membrane and propagated in mouse embryo tissues exhibited during the first few generations progressively increasing titers, whereas those started with the chick embryo tissue culture showed rapid diminution of potency.

The multiplication of the virus grown upon solid medium used here occurred exclusively in the tissue cells. This followed from the result of titrations in which tissue, washings of the agar surface after the removal of the tissue, condensation water, and different layers of the agar slant were used. It was found that whereas the tissue showed the titer of 1:100,000, washing prepared by using 5 cc of normal saline to an agar slant and condensation water gave weak

skin reactions when employed in the undiluted state. No reaction was elicited when ground agar removed from different depths of the slant was tested.

To determine the optimal time for incubation of cultures, a series of 7 tubes was prepared by inoculating chick embryo with an infected chorio-allantoic membrane. One tube was immediately placed in the icebox, and the remaining 6 tubes were incubated for 3, 4, 5, 7, 9, and 11 days, respectively. Titration of all of these cultures, performed upon the same rabbit, showed the titer of 1:1,000 for the first tube, and of 1:10,000, 1:10,000, 1:10,000, 1:1,000, 1:0 and 1:0 for the remaining 6 cultures. The experiment showed that the multiplication of the virus took place during the first 5 days. Deterioration of potency observed on further incubation was due, in all probability, to the effect of incubator temperature. This is suggested by the fact that cultures grown for 5 days and then kept in the icebox showed no decrease in their titer during the first 3 weeks of storage.

Summary. The cultivation of the vaccine virus in agar-slant tissue cultures was shown to be possible both with chick and mouse embryo tissues. The former was found to be distinctly superior so far as the growing qualities of the tissue are concerned.

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Toxic Manifestations After Oral Administration of Sodium Sulfapyridine.

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In 2 previous papers^{1, 2} we have reported the occurrence of uroliths consisting mainly of 2-(acetylsulfanilyl amino) pyridine, in rats, rabbits and monkeys following oral administration of sulfapyridine. Similar results were also published by Gross, Cooper and Lewis.³ Marshall, *et al.*,⁴ have shown that sulfapyridine in the form of its soluble sodium salt is more rapidly and completely absorbed than sulfapyridine, and have recommended its intravenous injection in

¹ Antopol, W., and Robinson, H., *Proc. Soc. Exp. Biol. and Med.*, 1939, **40**, 428.

² Molitor, H., and Robinson, H., *Arch. Internat. de Pharm. et Ther.*, in press.

³ Gross, P., Cooper, F., and Lewis, M., *Proc. Soc. Exp. Biol. and Med.*, 1939, **40**, 448.

⁴ Marshall, E. K., Bratton, A., and Litchfield, J., *Science*, 1938, 597.

patients in whom administration of sulfapyridine by mouth is impossible, or intestinal absorption is very poor, or prompt action of the drug is imperative.⁵ Since sodium sulfapyridine is excreted both as free and acetylated sulfapyridine it might be expected that its administration would result in a greater incidence of urolithiasis. The strong alkalinity of the compound also suggests the possibility of undesirable local effects depending upon the method of administration.

In the present paper we wish briefly to report the results of a series of experiments in which 10% aqueous solutions of sodium sulfapyridine (pH 11.4) were administered by stomach tube to 160 mice, 150 rats, 24 rabbits and 21 monkeys. Single doses of 3 and 4.5 g per kg produced gastric congestion and irritation in mice, rats and rabbits, as well as frequent urolithiasis in rats and rabbits. These phenomena became more pronounced after repeated administration (daily feeding for 10 days). In monkeys 0.5 g per kg or more produced gastric congestion with occasional erosions and marked urolithiasis in 8 out of 10 animals; 0.25 g per kg resulted in urolithiasis in 3 out of 5 animals, whereas no concretions were found with 0.1 g per kg. Rats fed doses of 2 and 3 g per kg lost weight and showed a general debility. Hematuria was frequent after the third day and all animals dying during the feeding period showed renal calculi and a severe hydronephrosis. Urolithiasis was also found in 13 out of 14 rats which survived the 10-day feeding period and were sacrificed on the 11th day. The degree of changes in the urinary tract of rats, rabbits and monkeys varied from mild dilatation of the ureters with little or no change in the kidney to an enormous dilatation of the ureter and kidney with partial disappearance of the kidney substance.

As is the case with sulfapyridine, no evidence of urolith formation was found in mice.*

Summary. Oral administration of sodium sulfapyridine produces marked gastric irritation and results in rats, rabbits and monkeys in the formation of urinary concretions. The minimal doses necessary to produce these phenomena vary from 0.25 g per kg in monkeys to 2 g per kg in rats, administered daily for 10 days. Larger doses (3 to 4.5 g per kg) may cause urolithiasis even after a single administration.

⁵ Marshall, E. K., and Long, P. H., *J. Am. Med. Assn.*, 1939, **112**, 1671.

* However, since submission of this paper we have been able to produce regularly urolithiasis also in mice by oral or intravenous administration of acetylated sulfapyridine in the form of its sodium salt.

Sensory Components of the Phrenic Nerve of the Cat.

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The presence of sensory components in the phrenic nerve was known even before Luscka¹ gave a fine description of the course and distribution of this nerve. Schreiber² described increases in blood pressure brought about by stimulation of the central end of the cut phrenic in dogs. In addition to the extensive treatment of the phrenic nerve by Felix,³ a number of papers have appeared in which functions of the sensory fibers in this nerve have been investigated: Capps and Coleman,⁴ Greene,⁵ Pollock and Davis,⁶ Hinsey and Phillips,⁷ Thornton,⁸ and Little and McSwiney.⁹

Ferguson's anatomical analysis¹⁰ consisted of removal of the 3-4-5-6 C dorsal root ganglia in a cat. After a degeneration interval, he found that one-third of the myelinated fibers had undergone degeneration. This procedure is open to the criticism that possibly he traumatized some of the ventral root fibers at the time the operation was done.

Instead of degenerating the sensory fibers in the phrenic nerve, we have left them intact and have degenerated the other nerve fibers. To accomplish this, the sympathetic trunk was removed from above the superior cervical ganglion down to the lower thoracic region and the ventral roots of C 2-3-4-5-6-7-8 cervical segments were sectioned. Suitable degeneration intervals intervened before the termination of the experiments. Three preparations were made as follows:

Cat No. 59. January 15, 1937. Removed right and left superior cervical sympathetic ganglia and the sympathetic chains in the neck down through the middle cervical sympathetic ganglia. February 5, 1937.

¹ Luscka, H., *Die Anatomie des Menschen*, I, Laupp and Siebeck, Tübingen, 1862.

² Schreiber, J., *Arch. ges. Physiol.*, 1883, **31**, 577.

³ Felix, W., *Deutsche Z. f. Chir.*, 1922, **171**, 397.

⁴ Capps, J. A., and Coleman, G. H., *An Experimental and Clinical Study of Pain in the Pleura, Pericardium, and Peritoneum*, Macmillan Co., New York, 1932.

⁵ Greene, C. W., *Am. J. Physiol.*, 1935, **113**, 399.

⁶ Pollock, L. J., and Davis, L., *Arch. Neurol. Psychiat.* 1935, **24**, 1041.

⁷ Hinsey, J. C., and Phillips, R. A., *Am. J. Physiol.*, 1937, **119**, 336.

⁸ Thornton, J. W., *J. Physiol.*, 1937, **90**, 85.

⁹ Little, M. G. A., and McSwiney, B. A., *J. Physiol.*, 1938, **94**, 2.

¹⁰ Ferguson, J., *Brain*, 1891, **14**, 282.

Removed right and left thoracic sympathetic chains from above stellate ganglia to below T 7. February 26, 1937. Cut ventral roots of left C 3-4-5-6-7 spinal nerves, March 15, 1937. Animal sacrificed with ether.

Cat No. 10. September 10, 1937. Cut left ventral roots of C 2-3-4-5-6-7-8 intra- and extradurally. October 8, 1937. Removed left sympathetic chain from above superior cervical ganglion down through T 8. November 1, 1937. Animal sacrificed with ether.

Cat No. 11. September 14, 1937. Cut left ventral roots of C 2-3-4-5-6-7-8 intra- and extradurally. October 8, 1937. Removed left sympathetic chain from above superior cervical ganglion down through T 8. November 1, 1937. Animal sacrificed with ether.

The removal of the postganglionic sympathetic neurons is important in this procedure because of the fact that anastomotic branches are contributed from the sympathetic chain at the level of the stellate ganglion and above. These branches may contain myelinated sensory fibers, and myelinated as well as unmyelinated postganglionic ones. While most of the sensory fibers from the phrenic of the cat enter the spinal cord at the level of the 5th and 6th cervical segments (Little and McSwiney⁹), there is a possibility that some may enter at the 4 C level. Our dorsal root sections were either one or two segments above and below the levels at which somatic motor fibers are thought to enter the phrenic nerve. In each case, the absence of a response on stimulation of the distal portion of the cut phrenic showed that no somatic motor fibers had escaped section. The degeneration intervals were such that there seems to be little possibility that regeneration confused the picture, particularly in Cat No. 59.

Histological studies were made on the portion of the phrenic between the heart and the diaphragm. Axis cylinders were counted in silver preparations (method of Bodian) and myelin sheaths in osmic acid preparations from adjacent portions of the nerve. The diameters of the myelinated fibers were determined by measurements on photomicrographs taken with considerable magnification.

In Fig. 1, the numbers of myelinated fibers are represented by dots and the sizes are expressed in microns on the abscissae. The numbers of nerve fibers in the normal right and left phrenic nerves of an animal are not identical and consequently a comparison of the number of sensory myelinated fibers in the left operated phrenic nerve with the total number in the normal right one has its limitations. However, some rough idea of the relative number can be obtained. The normal right phrenic nerve has somewhere in the neighborhood

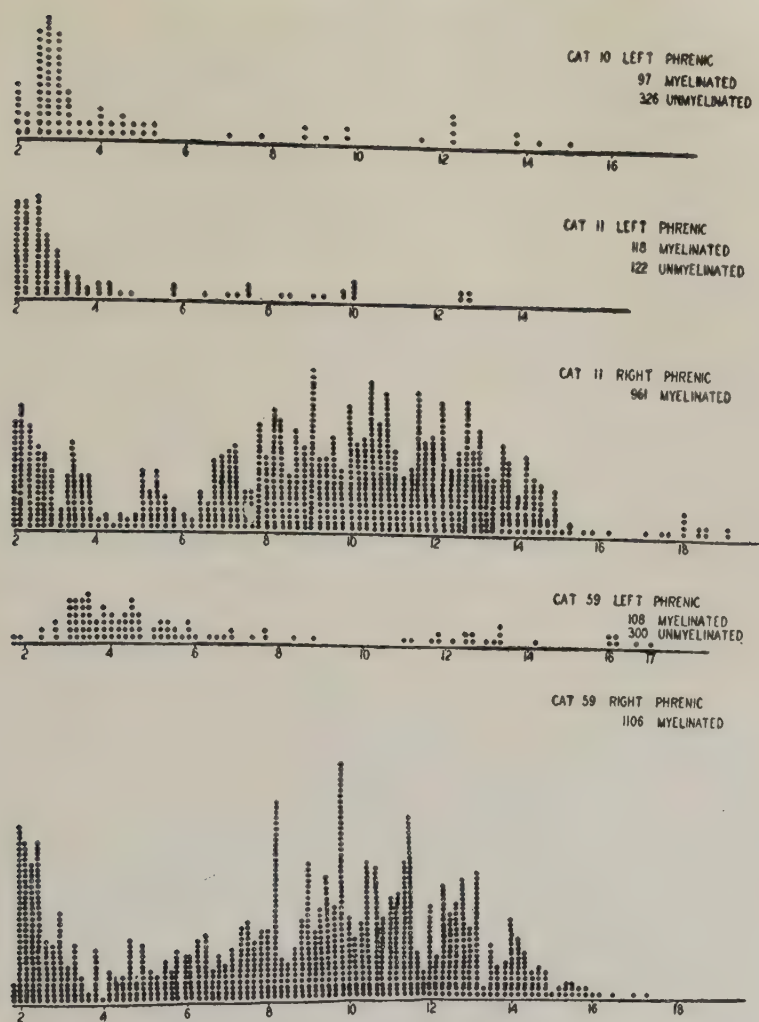


FIG. 1.

Analyses of myelinated fibers in phrenic nerves from Cats No. 10 (with only sensory fibers present), No. 11 and No. 59 (left phrenic with only sensory fibers and right one with all fibers present). Diameters of fibers represented in microns on abscissæ and each fiber by a dot on the ordinates.

of 1000 fibers and there are about 100 myelinated sensory fibers in the left operated phrenic. This indicates that approximately 10% of the myelinated fibers of the phrenic are sensory. This relationship is low when it is compared with that in a motor branch of the femoral nerve in which 30 to 40% of the myelinated fibers are sensory.

In cats No. 10 and No. 59, there were 3 unmyelinated sensory

fibers for every myelinated one while in Cat No. 11, the ratio was about one to one. However, the silver staining was more satisfactory in the first 2 than it was in the last one. A 3 to 1 ratio of unmyelinated to myelinated sensory fibers is high as compared to the one to one ratio determined by Ranson and Davenport¹¹ for a muscle branch of the femoral nerve. In such a comparison, it should be recalled that the portion of the phrenic nerve which has been analyzed contains sensory fibers to the central portions of the diaphragmatic peritoneum and pleura as well as the proprioceptive innervation to skeletal muscle. While the presence of muscle spindles in the diaphragm has been affirmed and denied (for review see Hinsey¹²), we have not found them in our preparations but we must admit that our search has not been extensive enough to deny their presence.

With the coöperation of Dr. Harry Grundfest of the Rockefeller Institute, action potentials were recorded from the left phrenic of Cat No. 11. The velocity of conduction in the fastest fiber was 63 m.p.s. and there was a slow wave, made up of potentials of fibers in which velocity of conduction was about 20 m.p.s. No "C" spike was observed.

Some of these sensory fibers which have been isolated are ones whose impulses are involved in the production of the sensation of pain from the central portion of the diaphragmatic peritoneum and pleura. This has been investigated by Capps and Coleman,⁴ Pollock and Davis,⁶ and Hinsey and Phillips.⁷

Summary. By degeneration of the somatic motor and sympathetic fibers in the phrenic nerve, it has been shown histologically that this nerve contains myelinated sensory fibers of different sizes and unmyelinated ones. The ratio of unmyelinated to myelinated sensory fibers is relatively high in 2 of the 3 phrenic nerves studied as compared to that reported for a motor branch of the femoral nerve.

¹¹ Ranson, S. W., and Davenport, H. K., *Am. J. Anat.*, 1931, **48**, 331.

¹² Hinsey, J. C., *Physiol. Rev.*, 1934, **14**, 514.

Simple Method of Measuring Brightness Threshold of Dark Adapted Eye at All Ages.

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The relation between the vitamin A status of the body and the eye's ability to adapt to the dark is now well established.¹⁻⁵ The chemical mechanism underlying this relation has been studied by Wald.⁶

While several instruments for measuring dark adaptation are available,⁷⁻¹⁰ none is applicable to infants and very young children. In the course of an investigation¹¹ of the vitamin A requirements of infants, necessity impelled us to devise a method for measuring their ability to adapt to the dark. Fortunately, our task was simplified by a number of recent studies of this function in adults. It has been demonstrated that whereas the dark adaptation of persons with certain diseases is more or less delayed,^{2, 12} alterations in the vitamin A status of clinically normal individuals are accompanied by a simple rise or fall of the visual threshold, the speed of adaptation remaining constant.^{3, 4, 5} Hence, where it is not possible or convenient to measure the complete time course of dark adaptation, it is sufficient for purposes of dietary studies of normal subjects to determine the final or equilibrium threshold alone.⁴ The present apparatus and procedure accomplishes this in a satisfactory and simple manner.

In measurements of the precise course of dark adaptation, fixation

* Dr. Charles Haig is now at the Research Division for Chronic Diseases, Department of Hospitals, New York City.

¹ Fridericia, L. S., and Holm, E., *Am. J. Physiol.*, 1925, **73**, 63; Tansley, K., *J. Physiol.*, 1931, **71**, 442; Wald, G., *J. Gen. Physiol.*, 1935, **18**, 905.

² Haig, C., Hecht, S., and Patek, A. J., Jr., *Science*, 1938, **87**, 534.

³ Hecht, S., and Mandelbaum, J., *Science*, 1938, **88**, 219.

⁴ Wald, G., Jeghers, H., and Arminio, J., *Am. J. Physiol.*, 1938, **123**, 732.

⁵ Booher, L. E., Callison, E. C., and Hewston, E. M., *J. Nutrition*, 1939, **17**, 317.

⁶ Wald, G., *J. Gen. Physiol.*, 1935, **19**, 351.

⁷ Jeans, P. C., Blanchard, E., and Zentmire, Z., *J. Am. Med. Assn.*, 1937, **108**, 451.

⁸ Feldman, J. B., *Arch. Ophthalm.*, 1938, **19**, 882.

⁹ Hecht, S., and Shlaer, S., *J. Opt. Soc. Am.*, 1938, **28**, 269.

¹⁰ Ferree, C. E., and Rand, G., *Science*, 1939, **89**, 223.

¹¹ Lewis, J. M., and Barenberg, L. H., *J. Am. Med. Assn.*, 1938, **110**, 1338.

¹² Patek, A. J., Jr., and Haig, C., *J. Clin. Invest.*, 1939, in press.

of the eye in relation to the direction of the stimulus is essential to insure that the portion of the retina measured is the same throughout the test. If only the final or equilibrium threshold of the most sensitive portion of the retinal periphery after complete dark adaptation is to be determined, fixation is unnecessary. Such a measurement is most simply accomplished by moving the test field of an adaptometer over the entire visual field on both sides and increasing its brightness until it is visible to the subject. The test may be made objective in children and adults by asking them to tell the direction from which they see the light, by turning off the light on occasion, and in a variety of ways which will occur to the operator.

We have made similar tests upon infants by utilizing the amazingly pronounced positive phototropism which they possess even for intensities close to the visual threshold of adults and children. The infant is placed upon its back and a tube of luminous paint (actually a radium paint pendant used on electric light chains) is attached to the center of the forehead with adhesive plaster. After 30 minutes of darkness the test field is held in the hand of the operator and moved slowly from side to side through 180 degrees of arc at a distance of about 10 cm from the eyes of the infant. When the test field is made sufficiently bright the infant sees it in the periphery of its visual field and turns its head in a corresponding direction. This movement is clearly indicated by the direction of motion of the luminous tube. The response is easily distinguishable from random movements.

Fig. 1 is a diagrammatic representation of the portable instrument† we have been using for such studies. No new principles of design are involved, its unique features being extreme portability and the fact that the test light unit is constructed so as to fit into the hands of the operator. The light from a 3.8 volt flashlight lamp *L*, operated at 0.28 amperes by battery *B* through a 6 ohm rheostat *R* and ammeter *A*, passes through a 1:10 neutral photometric wedge *W* and wedge balancer *WB*, diaphragm *D*₁, one of 4 neutral filters *F*, a violet glass filter *VF* (No. 511 Corning), and flashed opal glass *FO*. The latter is diaphragmed, *D*₂, to form a test field of 12 mm diameter, which at a distance of 10 cm from the eye subtends a visual angle of 7 degrees. The wedge and series of filters ride in a frame equipped with spring catches which hold them at the position selected by the operator, who manipulates them by means of the rods *C* which protrude from the light-tight metal housing *H*. The neutral filters regu-

† Machined and assembled by Mr. O. C. Rudolph, 55 Van Dam Street, New York City.

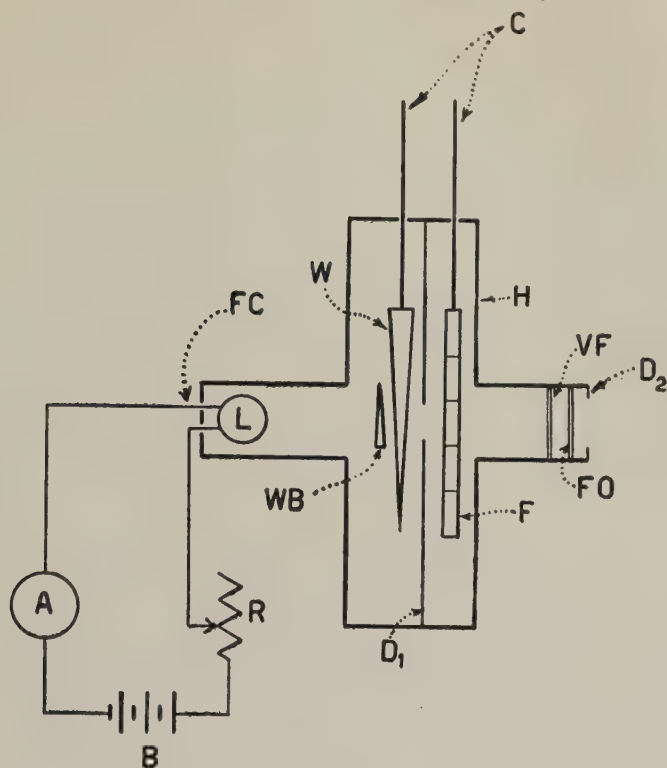


FIG. 1.

late the intensity in steps of one logarithmic unit (1:10). The wedge interpolates between any 2 filters in steps of 0.3 log units (1:2). The total range is just over 5 log units (1:100,000). The battery, ammeter, and rheostat form a unit which is conveniently placed out of the subject's view. This unit is connected by a flexible cord *FC* to the test light (with its appurtenances) held in the operator's hand. The entire apparatus is accommodated by a portable typewriter case.

The densities of the wedge and filters were measured by the method of Hecht, Shlaer, and Verrijp¹³ with a Martens polarization photometer, and a brightness determination for one setting of the wedge and filters made with a Macbeth illuminometer. The brightness unit adopted was the micromicrolambert (foot-candles $\times 1.076 \times 10^9$) expressed for convenience in logarithms. A table was prepared giving the brightness in these terms for each setting of the wedge and filters.

Violet light was adopted to provide a convenient differentiation between cone (violet-appearing) and rod (colorless) readings in

¹³ Hecht, S., Shlaer, S., and Verrijp, C. D., *J. Gen. Physiol.*, 1933, **17**, 237.

children and adults, to insure a degree of spectral purity, and because the rods, of primary interest in vitamin *A* estimations, are maximally sensitive to this part of the spectrum.

With children and adults it is possible to obtain an approximate measure of the speed of dark adaptation by exposing the eyes to a bright light for 2 or 3 minutes, following which observations of the rapidly descending threshold are made at frequent intervals until dark adaptation is relatively complete.

Measurements we have made of the visual thresholds of infants, children, and adults using this apparatus and procedure will be described elsewhere.

10695 P

Prolonged Administration of Cobra Venom in Relation to Kidney and Liver Function.

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The increasing therapeutic use of cobra venom as an analgesic in advanced malignant disease¹ and the extension of such clinical usage to other chronic painful conditions,^{2, 3} prompted the senior author to further pharmacological study of the effect of repeated administration of the drug for prolonged periods on certain vital physiological functions. The present communication describes the effect of repeated injection of large doses of cobra venom for long periods of time on the kidney and liver function of rabbits. A standard solution of the drug, prepared in these laboratories and assayed biologically to ensure a content of 5 mouse units of cobra venom per cc, was administered to a series of rabbits in doses of 5 or 10 mouse units daily 5 and sometimes 6 times a week. The results obtained in 10 such animals are exhibited in Table I. The weight of each rabbit, its kidney function, liver function and general condition were recorded at the beginning of the investigation. All the rabbits were kept on a liberal diet of rabbit food (Purina Chow) supplemented with fresh greens. In some rabbits the venom solution was injected intra-

¹ Macht, D. I., *Proc. Nat. Acad. Sc.*, 1936, **22**, 61.

² Macht, D. I., *Ann. Int. Med.*, 1938, **11**, 1824.

³ Rottmann, A., *Klin. Wchnschr.*, 1937, **16**, 1051.

TABLE I.
Effect on Kidney and Liver Function of Rabbits of Prolonged, Repeated Injection of Cobra Venom.

Rabbit	Time of Test		Weight in kilo		Kidney function 2 hours after		Liver function 15 minutes after		Total amount of cobra venom injected	
	Began	Ended	Initial	Final	Initial	Final	Initial	Final	cc	Mouse units
I	9-21-38	11-2-38	2.4	2.5	% 95	% 90	% 5-	% 5-	33	165
II	9-21-38	11-2-38	2.4	2.5	90	90	5-	5-	30	150
III	12-3-38	2-7-39	2.4	3.7	80	85	10	7	54	270
IV	12-3-38	2-7-39	1.5	2.7	80	80	7	5-	90	450
V	12-3-38	2-7-39	3.6	3.4	50	65	10	10	100	500
VI	1-17-39	5-8-39	3.8	3.2	80	80	6	0	209	1045
VII	2-6-39	5-8-39	3.2	3.0	80	85	7	5-	125	625
VIII	2-6-39	5-8-39	3.4	3.0	65	77	10	0	55	275
IX	2-6-39	5-8-39	3.5	3.4	60	70	6+	4	56	280
X	2-6-39	5-9-39	2.6	2.8	70	75	5	4	60	300

venously and in others intramuscularly, but the results obtained were the same regardless of method of administration. The dosage generally employed was 5 mouse units but some of the animals were injected with 10 mouse units. Inasmuch as 5 mouse units is the ordinary and 10 mouse units the exceptional therapeutic dose which the senior author recommends for human patients, such doses must be regarded as massive when considered in relation to rabbits weighing from 1.5 to 3 kg. Kidney function of the animals was measured by the phenolsulphonphthalein method with the Dunning colorimeter, and the urine was expressed exactly one and 2 hours, respectively, after injection of 6 mg of the dye in the ear vein. Liver function was determined by a modification of the Rosenthal⁴ method as follows: 5 mg of bromsulphalein per kilo weight of rabbit are injected intravenously, and a sample of blood is withdrawn from the heart precisely 15 minutes thereafter. In normal healthy rabbits the bromsulphalein content of the serum 15 minutes after its injection is usually not more than 5% and sometimes is too small to measure at all.

Results. Examination of the tabulated data shows that such injections were made in all the animals for a number of weeks and in some instances for several months. As a matter of fact, some of the animals are yet alive at this writing and still in good condition. Additional experiments on another series of rabbits are in progress. Nevertheless the data reveal that such administration of cobra venom impaired neither kidney nor liver function. Microscopic examination of the kidneys and liver of some of the animals, which were killed with ether, also revealed no pathological change. In fact, although the kidney and liver function of Rabbit V was definitely impaired at the outset, no damage was produced by the venom injections. Rabbit IV became impregnated in the course of the investigation, had a normal gestation and reared 2 of her 3 normal offspring, the third being accidentally killed after birth. This animal received nine intravenous and 81 intramuscular injections of cobra venom, a total of 90 cc or 450 mouse units of the drug. Rabbit VI received 40 intravenous injections of 10-mouse-unit doses, then 61 intramuscular injections of 10-mouse-unit doses, and finally 7 other injections of the ordinary dosage, or 5 mouse units of cobra venom. Rabbit VII received 17 intravenous and then 38 intramuscular injections of 5-mouse-unit doses of cobra venom. Rabbit IX received the same dosage in 18 intravenous and 38 intramuscular injections. The rest of the animals received only intramuscular injections. The

⁴ Rosenthal, S. M., and White, E. C., *J. Pharm. and Exp. Therap.*, 1924, **24**, 265.

histological findings as well as the results obtained in the author's studies on a new series of rabbits will be described in detail in another paper. In conclusion it may be stated that although thousands of doses of cobra venom have been injected by numerous physicians in different parts of the country, the authors have never received a single report describing any impairment of kidney function by this medication.

Summary. Large doses of cobra venom solution, 5 to 10 mouse units, equivalent to the ordinary and the exceptional dosage employed for adult humans, were administered to rabbits 5 or 6 days a week for periods ranging from 7 to 22 weeks. Functional tests made at the beginning and end of the investigation revealed that neither kidney nor liver function of any of the rabbits had been impaired during the whole course of experimentation.

10696

Relation of Suprarenal Hemorrhage to Loss of Vitamin C in Experimental Diphtherial Intoxication.

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The most characteristic lesion of acute experimental diphtherial intoxication in the guinea pig, other than the local disturbances at the site of injection, is the enlargement of the suprarenal glands with the accompanying congestion and hemorrhage, an extensive study of which was reported by Abramow.¹ Harde² found that the vitamin-C content of these organs was greatly diminished when death followed the injection of diphtherial toxin. Torrance³ confirmed this finding and reported that the injection of other toxic bacterial products, such as filtrates of the meningococcus, which give rise to congestion and hemorrhage of the suprarenal glands, cause the vitamin-C concentration to fall to similar low levels.

Since the essential tissue-changes in acute scurvy are associated with alterations in the capillary walls which result in generalized hemorrhage, it seemed not unlikely that the extravasation of blood

¹ Abramow, S., *Z. f. Immunitäts.*, 1912, I, **15**, 12.

² Harde, E., *C. r. Acad. de Science*, 1934, **199**, 618.

³ Torrance, C. C., *J. Bact.*, 1937, **33**, 645.

in the suprarenal glands is a manifestation of what might be called a "localized scurvy." This hypothesis is strengthened by the experiments described.

The ascorbic-acid content of the suprarenal glands of guinea pigs was determined by a modification of the method of Bessey and King.⁴ The hemoglobin-content was determined by extracting with 0.1 N hydrochloric acid the pulp obtained by grinding the tissue. Since the resultant turbid solutions of acid hematin could not be compared with the clear solutions obtained by diluting blood with the same acid, a series of standards was prepared from the suprarenal extracts themselves. Two solutions that represented the extremes of the available color range were selected and given arbitrary values of 100 units and 0. Intermediate steps in the series were attained by mixing the 2 in decimal proportions. The standards remained satisfactory for several weeks in the cold room.

Thirty-five pairs of suprarenal glands were obtained from guinea pigs that had been used for routine standardization tests of diphtherial toxin. Each gland was bisected; one half was used for the determination of the vitamin-C concentration and the other for the extraction of the hemoglobin. The correlation of the vitamin-C concentration and the hemoglobin was

$$r_{12} = -0.54 \pm 0.08.$$

This figure indicated a high degree of inverse association between the 2 substances.

Since the vitamin-C concentration of suprarenal tissue is higher than that of the blood, hemorrhage in these organs would, by dilution, reduce appreciably the concentration of ascorbic acid. The total ascorbic-acid content of the suprarenals of each of the animals was, therefore, calculated from the data available and its relation to the hemoglobin-concentration determined. The correlation-coefficient thus obtained was

$$r_{12} = -0.40 \pm .096$$

This figure, 4.2 times its probable error, is significant and indicates a degree of inverse relationship between the hemorrhage and the total vitamin C in the suprarenals at death.

Twelve guinea pigs weighing between 230 and 280 g were injected with from 0.8 to 1.2 minimal lethal doses of diphtherial toxin. Each of 10 similar animals received 100 mg of vitamin C and the same amounts of toxin. Two injected with 100 mg of vitamin C, 2 with 10 mg, and 2 uninjected guinea pigs served as controls. The animals that received toxin alone, and all but one of those given vitamin

⁴ Bessey, O. A., and King, C. G., *J. Biol. Chem.*, 1933, **103**, 687.

TABLE I.

Effect of Simultaneous Injection of Vitamin C and Diphtheria Toxin on Hemorrhage in Suprarenals of Guinea Pigs.

No. of animals	Material injected	Average hemoglobin units*	Stan. dev.
10	100 mg Vit. C + 0.8 to 1.2 M.L.D. toxin	23.5	± 8.9
12	0.8 to 1.2 M.L.D. toxin	77.5	± 32.5
6	Controls—no toxin	6.6	± 7.9

*Arbitrary units. 100, the greatest amount of Hb found in any suprarenal; 0, that in normal animal.

C and toxin died in less than 10 days. The 6 animals that did not receive toxin and the one that survived the toxin-vitamin injection were sacrificed. The suprarenals were removed. The right one was fixed in Zenker's fluid, the left was used for hemoglobin-determination. Since no significant differences were observed between the suprarenals of the uninjected animals and those that received vitamin C alone, the data are combined in Table I. The hemoglobin-content of the suprarenals of the guinea pigs injected with toxin alone was 2.8 times as great as that of the group that received toxin and vitamin C. These observations were confirmed by histological examination of the right suprarenals from the same animals.

Summary and Conclusions. An inverse correlation was demonstrated between the hemorrhage occurring in the suprarenals of guinea pigs injected with a fatal dose of diphtherial toxin and the total vitamin C remaining in these glands at death. When diphtherial toxin was administered simultaneously with a large dose of vitamin C, the hemorrhage of the suprarenals which customarily follows the injection of the toxin, was greatly reduced. These findings suggest that such hemorrhages follow the reduction in the vitamin-C concentration in this gland as they do elsewhere in the body, and thus result only indirectly from the action of the toxin.

Morphology and Nature of Pleuropneumonia-Like Microorganisms.*

L. DIENES AND E. R. SULLIVAN.

From the Department of Pathology and Bacteriology, and the Medical Clinic, Massachusetts General Hospital, and the Department of Medicine, Harvard Medical School.

We have recently isolated from the lungs of rats and mice 5 strains of pleuropneumonia-like microorganisms. The purpose of this note is to indicate that the morphology of these strains, like that of the L₁ strains of Klieneberger,¹ is essentially bacterial.

Most authors regard the organism of pleuropneumonia bovis and similar organisms as in a class distinct from bacteria or other well-known microorganisms. They have a characteristic morphology and colonial appearance. Especially in pathological tissues or exudates they are filterable, and the diseases which they produce are often mistaken for virus diseases. The microbes included by Klieneberger² in this group produce tiny colonies consisting of fine pleomorphic, very fragile granules, which later swell up to form large round bodies. The vacuolization and coalescence of these round bodies produce eventually a foam-like appearance of the colonies. Saprophytic strains cultivated by Laidlaw and Elford,³ and by Seiffert,⁴ have been included in the pleuropneumonia group on the basis of filterability and special morphology.⁵

One of us^{6, 7} has pointed out that Klieneberger's L₁ strain, isolated from the streptobacillus moniliformis, is but a variant of this bacillus. For it not only can be isolated regularly from this bacillus, but occasionally reverts to it, and it conforms essentially to bacterial morphology. In young colonies, fine bacterial filaments are present, containing deeply stained granules. Part of these forms degenerate, and part swell up to large round bodies. A similar process can be

* The expenses of this investigation were defrayed in part by the Commonwealth Fund. This is publication 30 of the Robert W. Lovett Memorial for the study of crippling disease, Harvard Medical School.

¹ Klieneberger, E., *J. Path. Bact.*, 1935, **40**, 93; 1936, **42**, 587.

² Klieneberger, E., *J. Hygiene*, 1938, **38**, 458.

³ Laidlaw, P., and Elford, W. J., *Proc. Roy. Soc.*, 1936, **120**, 292.

⁴ Seiffert, G., *Zbl. Bakl. I. O.*, 1937, **139**, 337.

⁵ Oerskov, J., *Zbl. Bakl. I. O.*, 1938, **141**, 232.

⁶ Dienes, L., *Proc. Soc. Exp. Biol. and Med.*, 1938, **39**, 365.

⁷ Dienes, L., *J. Infect. Dis.*, in press.

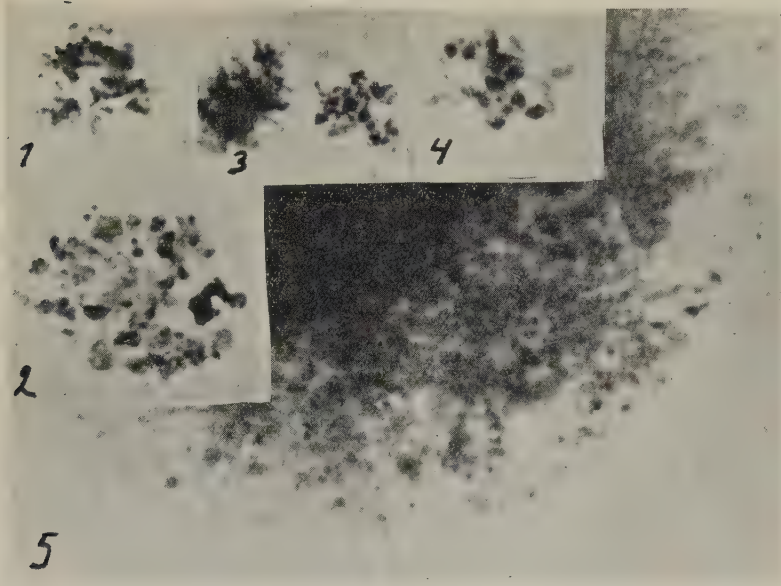


FIG. 1.

Photographs 1 and 2 represent colonies of the rat strain after 24 and 48 hours of incubation. Photographs 3 to 5 represent mouse strain 334. 3 and 4 illustrate young colonies with filaments and round bodies of different sizes. 5 shows the edge of a 48-hour colony consisting of large round bodies partly vacuolized.

Magnification (all photographs) is 1:2000.

observed occasionally with various bacteria, *i. e.*, colon or influenza bacilli. The L_1 strains differ from the other bacteria only by the regular occurrence of this phenomenon.

The morphology of the strains was studied by staining directly on the agar the developing colonies. Alkaline methylene blue or toluidin blue was used according to a technic previously described.⁷

The colonies of the rat strain remained very small (10 to 20 micra). They consisted at first of deeply stained pleomorphic granules. Following 24-48 hours of incubation, the granules swelled up to fairly large round or oval bodies. After repeated transfers the colonies grew larger.

The murine strains resemble more the L_1 organisms. In the young colonies there are fine curved filaments containing deeply stained granules. Even in the youngest colonies, the filaments are already partly transformed into rows of granules and round bodies. After 48 hours, the surface of the colonies is composed chiefly of large round bodies. These are often filled with tiny granules. After a longer period of cultivation some of the colonies grew fairly large (about 1 mm). In these the elements resembled regular bacterial forms

more closely than was the case immediately after isolation. But we did not observe a reversion to normal bacterial morphology as was observed in the case of the L_1 strains.

We were unable to verify the presence of fine non-bacterial filaments such as described by Klieneberger. No elements inconsistent with bacterial morphology were visible in the colonies of our strains.

10698 P

Diagnosis of Echinococcal (Hydatid) Disease in Man by Intradermal Reaction to Rabbit *Cysticercus* Antigen.

HARRY M. ROSE AND JAMES T. CULBERTSON. (Introduced by F.
P. Gay.)

*From the Departments of Medicine and of Bacteriology, College of Physicians
and Surgeons, Columbia University, New York.*

A skin test originally described by Casoni¹ and since modified by several investigators is generally considered to be the most reliable single guide to the presence or absence of suspected hydatid infection in patients.² The test is usually performed by injecting intradermally 0.1 to 0.5 cc of carbolized fluid obtained from a fertile hydatid cyst of either sheep or man. This fluid contains an antigen which maintains its potency for months. Both immediate and delayed skin reactions, consisting of wheals with pseudopods and surrounding erythema, have been described. The necessary hydatid fluid may be readily obtained in certain world areas, such as Southern Australia, where echinococcal disease is common in both man and animal. However, throughout North America the supply of cysts is small due to the rareness of the disease, and the clinician is often confronted with a diagnostic dilemma when the antigen is unavailable.

In the present communication observations are reported concerning an antigen which may be procured readily from cysticerci occurring in rabbits. Tests made thus far indicate that patients infected with echinococcus, or those who have recently had cysts removed, will give positive skin reactions with the antigen, whereas essentially

¹ Casoni, T., *Folia Clinica Chem. Micros.*, 1911-1912, 4, 5.

² For reviews of the literature, consult Taliaferro, W. H., *The Immunology of Parasitic Infections*, 1929, The Century Co., New York; and Culbertson, J. T., *Arch. Path.*, 1938, 25, 85, 256.

negative results are obtained in individuals who have never harbored the parasite. Cysticerci obtained from the rabbit are those of *Taenia pisiformis*, a cestode which is found naturally in that animal. Among stock laboratory rabbits the incidence of infection varies from 25 to 50%, being higher in the older animals.

Preparation of Antigen: The abdomens of healthy rabbits are open with sterile precautions and searched for cysts. When these are found they are removed to Petri dishes where the adventitial envelope, which is composed of rabbit tissue, is teased away with sterile dissecting needles. From 5 to 7 of the cysts are triturated in a sterile mortar, thus destroying the contained scolices, and are then extracted with 3 to 4 cc of sterile saline for 2 hours at 37°C. The resulting suspension is centrifuged to remove all heavy particles and portions are cultured aërobically and anaërobically to determine sterility. The fluid finally obtained is opalescent, but no sediment appears on standing.

Relationship of Rabbit Cysticercus Antigen to Specific Hydatid Antigen: Taxonomically the rabbit cysticercus is closely related to the echinococcus since both are cestodes of the genus *Taenia*. Furthermore, an antigenic relationship between these species has recently been demonstrated by Outeirino.³ In order to study this antigenic relationship several procedures were carried out. Blood serum from a patient who had recently had an echinococcal cyst removed was found to give a delayed precipitin-reaction with the rabbit antigen as well as with the fluid removed from the hydatid cyst itself. Furthermore, both antigens partially fixed alexin in the presence of the aforementioned serum. Finally, rabbits which proved to be infected with *Taenia pisiformis* all gave a skin reaction of the delayed type not only with the rabbit antigen but also with the human echinococcal cyst fluid. The positive reactions appeared in 24 to 48 hours and consisted of erythematous, indurated areas from 1 to 2 cm in diameter. Rabbits in whom autopsy failed to disclose the parasite invariably had negative skin tests.

Skin Tests in Known Cases of Hydatid Disease and in Normal Subjects: Four patients known to be suffering from hydatid disease were skin tested with both the rabbit and the human cyst antigens. Normal saline and rabbit serum diluted 1-10 were used as controls. Intradermal injections were made on the volar surfaces of both forearms, using 0.1 cc of each test material. All of the patients exhibited a prompt and vigorous reaction to both antigens, whereas the saline and rabbit serum controls were uniformly negative.

³ Outeirino, J., *Ann de méd.*, 1935, **38**, 493.

The positive reactions were characterized by wheals which began to form in less than 5 minutes and reached their maximal size in from 15 to 30 minutes. The wheals varied from 2 to 4 cm in diameter, with large pseudopods and usually some itching. A surrounding zone of erythema from 5 to 10 cm in diameter was noted. Fading of the reactions was complete in about 12 hours although residual induration persisted for from 24 to 36 hours. The reaction to the rabbit antigen was invariably more marked than that with the echinococcal cyst fluid, probably due to the fact that the former represented a more concentrated antigen.

As a control group, 10 normal men and 10 normal women were also skin tested. In several of these individuals both antigens produced wheals up to 1 cm in diameter with some erythema, but no pseudopods or subjective itching appeared. These slight reactions faded promptly, leaving no induration.

It must be noted that skin reactions resulting from the intradermal injection of antigenic extracts obtained from members of the genus *Tænia* are fundamentally group-specific rather than species-specific. Therefore, in the diagnosis of hydatid disease by the methods described in this paper, a careful stool examination is essential in order to exclude positive skin reactions due to infection with intestinal cestodes such as *Tænia saginata*.

Summary. An extract of cysticerci of the common rabbit cestode, *Tænia pisiformis*, has been used as an antigen for skin testing in cases of echinococcal infection. An antigenic relationship between *Tænia pisiformis* and *Tænia echinococcus* is suggested by the precipitin-reaction, the alexin fixation, and the skin reaction in rabbits harboring *Tænia pisiformis*.

Four patients known to have hydatid disease gave immediate positive skin reactions with the rabbit antigen. Twenty normal subjects used as controls exhibited at most but slight reactions, and were considered negative.

Effect of Progesterone and Other Hormones on Liver Glycogen.*

ROBERT GAUNT, JOHN W. REMINGTON AND ABRAHAM EDELMANN.

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The carbohydrate stores of intact fasting animals can be raised by large doses of cortical hormone.^{1, 2} It has recently been shown that progesterone maintains the lives of adrenalectomized animals.³⁻⁹ Since the normal corpora lutea of pseudopregnancy will maintain life after adrenalectomy, this cortin-like activity is probably of physiological significance at least in certain species (references elsewhere³).

We report here studies to determine if progesterone and other sex hormones have an action like cortical hormone on carbohydrate metabolism as judged by the effects of acute overdosage on the glycogen stores of fasting animals. We used the ferret in initial experiments because it seems to be in other respects an unusually responsive species to the cortical hormone-like action of progesterone.

In Ferrets. All experimental ferrets were fed for one week prior

* This work was aided by a grant from the Penrose Fund of the American Philosophical Society.

The authors are indebted to Professor C. N. H. Long and Dr. Jane Russell for advice concerning this investigation.

The hormones used here were generously supplied us as follows: Progesterone by Dr. Erwin Schwenk, Schering Corporation; cortical extracts (Esechatin) by Dr. Oliver Kamm, Parke, Davis and Co.; testosterone propionate (Oreton) by Dr. Max Gilbert, Schering Corporation; Stilboestrol and pregnancy urine extract (Follutein) by Dr. J. A. Morrell, E. R. Squibb and Sons; and pregnant mare serum (Gonadin) by Mr. Donald Wonder, Cutter Laboratories.

¹ Britton, S. W., and Silvette, H., *Am. J. Physiol.*, 1932, **100**, 693.

² Katzin, B., and Long, C. N. H., *Proc. Am. Physiol. Soc.*, 1938, p. 113; and 1939, p. 135.

³ Gaunt, R., and Hays, H. W., *Am. J. Physiol.*, 1938, **124**, 767; and Gaunt, R., Nelson, W. O., and Loomis, E., *Proc. Soc. Exp. Biol. and Med.*, 1938, **39**, 319.

⁴ Fischer, A., and Engel, M., *Lancet*, 1939, **236**, 354.

⁵ Thorn, G. W., personal communication.

⁶ Greene, R. R., Wells, J. A., and Ivy, A. C., *Proc. Soc. Exp. Biol. and Med.*, 1939, **40**, 83.

⁷ Bourne, G., *J. Physiol.*, 1939, **95**, 12p.

⁸ Schwabe, E. L., and Emery, F. E., *Proc. Soc. Exp. Biol. and Med.*, 1939, **40**, 383.

⁹ Corey, E. L., personal communication.

to experimentation on weighed and identical portions of Ken-L-Ration, fresh ground meat and fresh milk. Feeding was in such amounts as to permit a weight gain of from 5-10 g per day. All food was withdrawn for the last 24 hours of the experiment. The females were in early but not full estrus if not designated as pseudopregnant. Pseudopregnancy was produced by injections of pregnant mare serum and pregnancy urine extracts. All animals were about 10 months old. Sixty mg of progesterone was given in sesame oil in 5 divided doses beginning 36 hours and ending 8 hours before sacrifice. In the cortical hormone series, 20 cc of cortical extract in sesame oil was given from 36-12 hours before the experiment ended, and 30 cc of aqueous extract was given during the last 12 hours before sacrifice. Controls received sesame oil and saline in amounts similar to the volumes of these solvents used in the cortical extract-treated series. Control and treated cases were run simultaneously.

The results are shown in Table I. It can be seen that both cortical extract and progesterone elevated liver glycogen above the level found in controls while muscle glycogen varied little if any. This was particularly noticeable in pseudopregnant animals which had been under the influence of their own corpora lutea for 2 to 3 weeks

TABLE I.
Ferrets.

Index No.	Sex	Liver Glycogen g %	Liver Glycogen mg % per 100 g body wt	Muscle Glycogen g %
Controls—Untreated, 24-hour fast.				
25	Male	.330	37.1	.452
26	"	.461	35.6	.463
34	Female	.158	30.0	.380
33	"	.189	28.2	.427
Av.		.285	32.7	.430
Given 50 cc Cortical Extract, 24-hour fast.				
29	Male	.798	121.0	.594
30	"	.924	125.7	.513
35	Female	.784	156.9	.520
36	"	1.002	195.7	.552
Av.		.877	149.8	.545
Given 60 mg Progesterone, 24-hour fast.				
27	Male	.781	96.4	.500
28	"	.795	98.2	.551
31	Female	.824	159.0	.468
32	"	.590	100.0	.464
Av.		.748	113.4	.496
Pseudopregnant—Given 60 mg Progesterone, 24-hour fast.				
20*	Female	1.696	261.0	.546
23	"	0.818	143.5	.621
24	"	1.424	219.1	.545
Av.		1.313	207.9	.571

* Adrenalectomized.

and then given 60 additional mg of progesterone as in other cases. The effect is not through the adrenals as shown by No. 20, an adrenalectomized animal, which had been maintained by her corpora lutea without any cortical hormone for 3 weeks.

Blood sugar figures were generally higher in treated animals. There were indications of increased serum ketones and urine nitrogen after treatment. No significant differences in blood dilution (hematocrit), or serum Na, K, or Cl were found.

In Rats. Experiments were done similarly on 125 g male rats with the exception that a 12-hour fast was used and injections of the sex hormones were begun 48 hours before sacrifice. Cortical hormone was given as follows: 6 cc in oil from 36-12 hours, and 10 cc in hourly divided doses during the last 10 hours before sacrifice.

The liver glycogen and blood-sugar raising effect of cortical hormone as previously reported in rats was confirmed (Table II). If the sex hormones had any effect it was only slight and not significant in this small series. Pending further study these data on rats are presented to show only that the rat does not respond to any of the sex hormones, quantitatively at least, as does the ferret to progesterone.

In a susceptible animal such as the ferret apparently a cortical hormone-like action of progesterone on carbohydrate metabolism can be demonstrated. The possible physiological significance of this fact is being studied further. The weak action of progesterone, if any at all, on this process in the rat may perhaps be correlated with the fact that the rat demands relatively enormous amounts of progesterone for life-maintenance after adrenalectomy, and that progesterone is of no assistance in this species to the stress of water intoxication,³ muscle work,¹⁰ and lactation¹¹ following adrenalectomy.

TABLE II.
Male Rats.

	No. rats used	Liver Glycogen g % Mean	Muscle Glycogen g % Mean	Blood Sugar mg % Mean
Controls—Untreated, 12-hr fast	9	0.335	0.441	76.3
Given 16 cc Cortical Extract, 12-hr fast	3	1.061	0.498	119.3
Given 35 mg Progesterone, 12-hr fast	3	0.337	0.457	92.8
Given 50 mg Testosterone Propionate, 12-hr fast	3	0.292	0.429	87.7
Given 35 mg Stilboestrol, 12-hr fast	6	0.443	0.463	96.0

¹⁰ Ingle, D. J., *Proc. Am. Physiol. Soc.*, 1939, p. 127.

¹¹ Tobin, C. E., personal communication.

Professor C. N. H. Long and collaborators have also been unable to demonstrate an effect of progesterone on the carbohydrate metabolism of rats.¹² Experiments carried out in a different fashion might have given positive results. Gilder and Phillips¹³ have found that estradiol-treated rats demonstrated significantly elevated liver glycogens only if glucose-fed.

Summary. Progesterone as well as cortical extract raised the liver glycogen levels of intact fasting ferrets. Pseudopregnancy probably enhanced the effectiveness of progesterone. In a small number of rats cortical extracts had similar effects, while progesterone, testosterone propionate and stilboestrol gave little if any response.

10700 P

Hormonal Induction of Abortion.*

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The present experiments represent an attempt to determine whether or not pregnancy may be interrupted in the rabbit by the induction of ovulation during the second trimester of gestation.^{1, 2, 3} It was prompted by earlier work⁴ upon animals observed during the last trimester of pregnancy in which it had been found that the duration of gestation could be profoundly altered by the induction of ovulation with extract of urine of pregnant women (Antuitrin S). The onset of parturition was delayed long past term in certain animals, while in others labor was induced prematurely. Factors which determined whether pregnancy would be lengthened or shortened were (1) the stage of pregnancy at the time of induction of ovulation, (2) the dosage of pregnancy urine extract adminis-

¹² Personal communication.

¹³ Gilder, H., and Phillips, R. A., *Proc. Am. Physiol. Soc.*, 1939, p. 86.

* This investigation was supported by grant from the Committee for Research in Problems of Sex, National Research Council.

¹ Wislocki, G. B., and Goodman, L., *Anat. Rec.*, 1934, **59**, 375.

² King, J. L., *Am. J. Physiol.*, 1938, **122**, 455.

³ Engle, E. T., and Mermod, C., *Am. J. Physiol.*, 1928, **85**, 518.

⁴ Snyder, F. F., *Bull. Johns Hopkins Hosp.*, 1934, **54**, 1.

tered, and (3) the parity of the animal. In the present experiments two of these factors were kept constant, namely, dosage of urine extract and parity of the animal, while the third factor varied, namely, the stage of pregnancy at the time of injection.

Accordingly, one series of animals was injected 11 days after mating, *i. e.*, at the beginning of the second trimester of pregnancy, while a second series was injected about 17 days after copulation, *i. e.*, near the middle of pregnancy. All animals received a standard dose of 10 rat units of Antuitrin S of Parke, Davis and Company, *i. e.*, approximately 3 rat units per k, diluted with water to a volume of 1 cc and administered by a single injection in an ear vein. Of the total of 40 rabbits upon which the present observations are based, 35 animals were primigravidæ. The duration of pregnancy averaged 32 days in this stock. The stage of pregnancy was accurately known since the animals were mated in the laboratory.

There was a striking difference in the course of pregnancy between the group of animals which was injected 11 days after mating and the second group which was injected 17 days after coitus. In the former group pregnancy continued to term; in contrast, in the latter group early interruption of gestation involved the entire litter in most of the animals and part of the litter in the others.

In the series of 12 animals injected at 11 days there was no evidence of interruption of pregnancy. Normal fetuses were delivered at term in 8 animals; in 3 animals which were sacrificed at 15 days in order to examine the ovaries, the fetuses were found alive; and in one animal birth of normal fetuses occurred at 30 days.

In the group of 28 rabbits which were injected about 17 days after mating, early interruption of pregnancy involved the entire litter in 18 animals. The stage of development of the fetuses showed that intrauterine death had occurred about the second or third day after the injection. Of the remaining 10 litters, 3 were examined within 48 hours after the injection and showed 16 dead fetuses and 6 living ones; in each of the 3 litters at least two-thirds of the fetuses had succumbed after the time of injection. Four litters examined 4 to 6 days after injection showed 14 living and 9 dead fetuses. There were at least 2 living fetuses in each of these litters but the dead fetuses had succumbed after the injection as shown by the stage at which their development had been arrested. Of 3 litters in which fetuses survived 4 days or longer following injection, no evidence of induced ovulation was found in 2 of the animals when the ovaries were examined microscopically; in the third animal, fresh corpora lutea were present.

The presence of fresh corpora lutea following injections was determined by microscopical examination of the ovaries. In the group injected at 17 days, fresh corpora lutea were seen in all cases except the 2 animals previously mentioned. In the group injected at 11 days, ovaries of only 3 animals were examined 4 days following injection at which time fresh corpora lutea were readily identified.

The striking difference in the course and outcome of pregnancy, depending upon the stage of gestation at which ovulation is induced, is indicative of the rate at which changes occur in the hormonal mechanism by which intrauterine life is maintained.

Summary. Ovulation was induced during pregnancy by a single intravenous injection of 10 rat units of pregnancy urine extract. Abortion commonly occurred in rabbits injected about the middle of pregnancy. On the other hand, in animals injected at the beginning of the second trimester, interruption of gestation did not occur.

10701

Viral Effect Produced by Intestinal Contents of Normal Mice and of Those Having Spontaneous Encephalomyelitis.

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Spontaneous encephalomyelitis of mice was first described by Theiler¹ as a new viral disease. The case incidence among stock Rockefeller Institute mice was shown to be 1 or 2 per 1,000² and its existence in Germany³ and in Japan⁴ was reported later. Interest in the malady lies in its similarity in many characters to poliomyelitis^{1, 4}, especially in size of the virus; in its action chiefly on the CNS and the naturally occurring characteristic flaccid paralysis, and in the pathological changes of the CNS. It has been called "poliomyelitis of mice," although Theiler¹ first demonstrated that there is no relationship between the two in host-susceptibilities and immunological reactions.

* I wish to acknowledge with thanks the valuable cooperation of Dr. M. Theiler of the International Health Division of the Rockefeller Foundation.

¹ Theiler, M., *Science*, 1934, **80**, 122; *J. Exp. Med.*, 1937, **65**, 705.

² Sabin, A. B., and Olitsky, P. K., *J. Exp. Med.*, 1938, **67**, 201.

³ Gildemeister, E., and Ahlfeld, I., *Cent. Bakt.*, I Abt., Orig., 1938, **142**, 144.

⁴ Iguchi, M., *Kitasato Arch. Exp. Med.*, 1939, **16**, 56.

In view of the recent discussion on the presence of virus in the feces in poliomyelitis, it was thought desirable to study the intestinal contents of mice having the Theiler disease-syndrome and of normal mice. One of 5 stock, "normal" mice caged together exhibited circling. After 4 days it was killed† and its brain and intestinal tract were removed. 0.03 cc of 10% broth suspension of the brain was injected intracerebrally into each of 4 mice, none of which developed disease. The intestines along with their contents were ground, and 1.75 g diluted with 14 cc broth. This was spun at 2500 rpm for 10 minutes and the supernatant filtered through a Berkefeld "V" candle. One of 4 mice injected intracerebrally with the filtrate developed, after 12 days, flaccid paralysis of both posterior extremities and of the lumbar muscles, and showed the usual signs of Theiler's disease. The transfer from this mouse and later passages to the 6th are tabulated. Further transfers are still being successfully carried out.

The source of material for these positive transmissions was a

TABLE I.
Serial Passages (to 6th Transfer) of Unfiltered Brain or Filtered Intestinal Material. Each mouse given 0.03 cc intracerebrally.

Passage	Mouse No. and history	Material tested	No. of mice used	No. paralyzed
1	1—circling, 4 days	Brain	4	0
		Intestines + contents	4	1
2	2-16—paralyzed, 2 days	Brain	4	4
3	2-21 " 2 "	Brain Intestines + contents	6	6
	2-22 " 2 "		5	3
	(pooled)			
4	2-25 " 1 "	Brain Intestines + contents	6	5
	2-39 " 1 "		8	4
	(pooled)			
5	2-45 " 2 "	Brain	4	2
		Intestines + contents	6	6
	2-46 " 7 "	Brain Intestines + contents	4	3
	2-47 " 7 "		6	3
	(pooled)			
6	2-59 " 3 "	Brain	4	1
		Intestinal contents alone	8	4
	2-63 " 3 "	Intestinal walls	8	0
	(pooled)			

† The lethal agent was ether; in operative procedures ether anesthesia was used.

mouse with an indefinite sign, *i. e.*, circling, but no paralyses or other disabilities. The next experiment was performed with the pooled brains, and pooled intestinal contents of 3 animals showing flaccid paralysis for 1 to 2 days. These mice represented the 2nd intracerebral passage of brain tissue derived from the naturally occurring, spontaneous disease. Here again the brain and the intestinal contents injected intracerebrally in normal mice induced the characteristic encephalomyelitis.

The lesions produced in the CNS by injection of filtered intestinal contents derived from both of the passage series are similar to those seen in the natural, spontaneous disease:¹ Neuronal necrosis and neuronophagia, especially marked in the cord, associated with typical perivascular infiltration and with little or no meningeal reaction. Moreover, mice which have recovered from the acute stages of the disease induced by intracerebral passage of brain derived originally from the malady in nature, are resistant to intracerebral inoculation of the "intestinal" active agent. Conversely, the majority of those which have failed to react to the latter are refractory to similar injection of brain obtained from the passaged natural disease.

Further experiments carried out to this time (in 1 or 2 tests comprising 8 to 10 mice in each) indicate that suspensions of intestinal walls washed completely free of their contents, mesenteric glands, salivary glands, and nasal mucosæ, derived from mice in the early stages of the affection produced by intracerebral inoculation of intestinal contents, fail to produce encephalomyelitis. In addition, suspensions of intestinal contents which were active when given intracerebrally exhibited no disease-producing effect when injected subcutaneously (abdominal area) nor sub- and intracutaneously (plantar tissue), and feeding the suspensions through a stomach-catheter or cannibalism of infected mouse-brain also failed to cause illness. Nor did animals reveal the disease when their plantæ were repeatedly scarified while kept in close contact with those in the active stages of encephalomyelitis. The purpose of these preliminary experiments was to elucidate, if possible, the way in which the virus might spread among mice and the relation thereto of the active agent in the intestinal contents, especially since the latter was, at times, as effectively disease-producing by intracerebral injection as were the brain and cord of infected animals.

Attention was then directed to a study of the intestinal contents of normal mice. It was found that the pooled brains of 6 stock mice (of the same age as those used heretofore, namely 30 days of age) showed, after intracerebral inoculation, no viral effect in 6

fresh mice, but the filtered pooled intestinal contents of the same stock animals, similarly inoculated, induced in 6 of 8 mice the characteristic encephalomyelitis. Intracerebral passage of the brain and filtered intestinal contents of the latter was again positive. It would now appear that (a) either normal mice harbor this virus in the intestinal contents, or (b) these contents of mice in general are non-viral but can activate, after intracerebral inoculation of filtrates, a latent carriage of virus, thereby inducing clinically apparent disease. If this occurs, one may postulate that the first test-mouse which reacted to inoculation of the filtered intestinal contents and thus initiated the present series of transmissions may have had its infection brought about in this way. Other substances introduced into the brain of mice do not, as a rule, cause this effect in view of the extremely low incidence of the malady observed among thousands of mice used in this laboratory for experimental transmission of various agents.²

10702

Observations on the Mode of Action of Sulfapyridine on *Pneumococcus*.*

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The introduction of successful chemotherapeutic measures against infection gives rise to speculations as to the mode of action involved. Whitby¹ reported on the effectiveness of sulfapyridine against certain types of the pneumococci and stated that "It appears to exert a definite action on the capsule of the pneumococcus." This view has not been accepted by those who have worked extensively with the drug, although the conception that the capsule is injured in some way, so that typing is either not possible or is rendered difficult after administration of sulfapyridine, has been retained by some clinicians.

Long² and his associates have expressed the view that the drug

* This investigation was supported in part by a grant from Hynson, Westcott & Dunning, Baltimore, Maryland.

¹ Whitby, L. E. H., *Lancet*, 1938, **1**, 1210.

² Long, P. H., Bliss, E. A., and Feinstone, W. H., *Penn. Med. J.*, 1939, **42**, 483.

"brings changes in the morphology and somatic elements of the pneumococcus and, in certain instances, so injures these micro-organisms that they are susceptible to phagocytosis by the monocytes and clasmotocytes present in the peritoneal exudate."

In the concentration of the drug used in his experiments, Fleming³ reported that sulfapyridine was bacteriostatic but not bactericidal and that leucocytes were necessary for the destruction of pneumococci and streptococci in blood containing sulfapyridine.

The following experiments were made in a further attempt to determine the manner in which sulfapyridine affects the pneumococcus or stimulates the body defenses against invasion by that micro-organism.

I. *Effect of Sulfapyridine on the Pneumococcal Capsule.* Mice weighing approximately 20 g were given 0.5 cc of a suspension containing 10 g of sulfapyridine in 100 cc of 10% gum acacia by mouth as described by Feinstone, Bliss, Ott and Long.⁴ The first dose was given in the evening, and in the morning (about 8 hours later) a second dose was administered. This was followed immediately by an intraabdominal injection of 0.5 cc of a 1-1000 dilution of a 14-hour broth culture of pneumococcus Type I. Peritoneal taps were made on each mouse at once after injection of the organisms, and each hour for 12 hours, and at 6-hour intervals thereafter until 36 hours had passed. During this time a satisfactory blood level of sulfapyridine was maintained in the mice by oral administration of the drug every 4 hours. This amount and method of administration was established as adequate by Long and Feinstone.⁵

Capsule-stains were made by the Hiss technic and the pneumococci present were typed by the Neufeld method from the material obtained by peritoneal tapping. In each case the capsule was found to be unaltered and the type of the organism, as determined by marked capsular swelling in the presence of homologous immune serum, was not changed.

Identical results were obtained when 0.5 cc of a 100 mg % suspension of the drug was injected into the animal's peritoneal cavity.

The experiment was repeated with Type III pneumococcus with similar results.

³ Fleming, A., *Lancet*, 1938, **2**, 564.

⁴ Feinstone, W. H., Bliss, E. A., Ott, E., and Long, P. H., *Bull. Johns Hopkins Hosp.*, 1938, **62**, 565.

⁵ Long, P. H., and Feinstone, W. H., *Proc. Soc. Exp. Biol. and Med.*, 1938, **39**, 486.

The same culture was inoculated into infusion-broth containing about 2% sterile ascitic fluid and 10 mg % sulfapyridine. As soon as the growth became visible, capsule-stains were made and the organisms showed a positive "*quellung*" reaction. Over a period of 24 hours no apparent change occurred in the capsules and no difficulty was encountered in typing by Neufeld's method.

II. *Effect of Sulfapyridine on Phagocytosis.* Sterile peritonitis was induced in a group of mice weighing 18-20 g each by injection of 2.0 cc of a suspension of 1% aleuronat and 1% gum tragacanth. Sulfapyridine was administered to these mice at 4- to 6-hour intervals, *per orum* as in the preceding experiment. Eight hours after injection of the foreign matter intraäbdominally and immediately following the second dose of sulfapyridine, the mice were given intraäbdominal injections of a 1-1000 dilution of a 12-hour broth culture of pneumococcus Type I.

Peritoneal taps were made at 2-hour intervals for 36 hours and the blood level of sulfapyridine was maintained by continued oral administration of the drug at the stated intervals.

Duplicate smears made from the peritoneal fluid were stained with Wright's and with Gram's stain. In these smears evidence of phagocytosis was looked for as well as careful examination of the cells for any visible somatic or morphological changes. A second group of mice was treated in the same manner except they were given no sulfapyridine and a third group was given a single intraäbdominal injection of 1500 units of Type I pneumococcus antiserum. The fourth group of mice received an intraäbdominal injection of 0.5 cc of 100 mg % sulfapyridine and 1000 units of antiserum.

There was no significant difference found in the numbers of leucocytes acting as phagocytes nor the number of bacteria engulfed by the phagocytes in the smears taken from the mice receiving sulfapyridine and those "unprotected" control mice. The mice in the groups that received antiserum showed a high percentage of leucocytes acting as phagocytes and the phagocytes contained large numbers of bacteria.

It was noted that what phagocytosis took place in the exudates from the control and sulfapyridine-groups occurred almost exclusively in the monocytes while in the groups getting antiserum the polymorphonuclear leucocytes also acted as phagocytes.

An increase in the number of pneumococci in the peritoneal exudate was observed in the control group of mice beginning with the

first tap and increasing with each succeeding examination. All mice in this group were dead in less than 20 hours.

The number of bacteria in the exudate from the mice getting sulfapyridine increased slightly during the first 6 hours but decreased rapidly after that. It was with extreme difficulty that any pneumococci were found either intra- or extra-cellularly after 10 hours, none being found after 18 hours. These mice survived until 3 or 4 days after the final administration of the drug. At autopsy pneumococci were recovered in large numbers from the peritoneal exudate and from the heart-blood.

In the mice treated with immune serum there was rapid ingestion of the diplococci. All organisms found after the fourth hour were intracellular and these gradually disappeared. These mice survived.

To determine the effect of sulfapyridine on the phagocytosis of pneumococci *in vitro*, tests were made using mixtures as indicated in Table I. The leucocytes were taken from guinea pigs after inducing a sterile peritonitis in the usual manner. The culture of pneumococcus Type I was grown for 15 hours in infusion-broth containing ascitic fluid. The sulfapyridine was added to give a final concentration in the mixture of 10 mg %. The volumes were made equal by the addition of physiological saline. The mixtures were incubated at 37°C for 30 minutes and except in tubes 1 and 2, smears made at that time. In tubes 1 and 2 either leucocytes or bacteria were added, as indicated, and incubated for another 30 minutes before making the smears. The slides were stained by Wright's and Gram's technic.

The results of this experiment indicated that phagocytosis was not enhanced by the presence of sulfapyridine. No appreciable in-

TABLE I.

Phagocytic Mixtures	Avg No. of pneumococci in 100 leucocytes	% of leucocytes acting as phagocytes
Leucocyte suspension, Sulfapyridine 10 mg %, Salt soln. (incubated 30' at 37°C.) Pneumococci added. Re-incubated	3.0	2.0
Pneumococci, Sulfapyridine 10 mg %, Salt soln. (Incubated 30' at 37°C.) Leucocytes added. Re-incubated	3.2	4.5
Leucocyte suspension, Pneumococci, Sulfapyridine 10 mg %, Salt soln.	3.1	3.5
Leucocyte suspension, Pneumococci, Immune serum, 0.1 cc—300 units, Salt soln.	240	60
Leucocyte suspension, Pneumococci, Immune serum, Sulfapyridine, Salt soln.	230	62
Leucocyte suspension, Pneumococci, Salt soln.	2.9	1.2

crease in the number of leucocytes acting as phagocytes or the number of bacteria engulfed by the phagocytes was noted in those mixtures containing sulfapyridine alone and the control containing none of the drug. In smears made from mixtures containing immune serum considerable phagocytosis was found.

III. *Bacteriostatic and Bactericidal Effect of Sulfapyridine.* Infusion-broth with ascitic fluid containing 10 mg % of sulfapyridine and similar media without the drug were inoculated with approximately 50,000 organisms from a 12-hour broth culture of pneumococcus Type I, as determined by plating on infusion-agar containing 10% defibrinated rabbit blood. Di-hourly plates were made and smears prepared and stained by Gram's stain, Hiss capsule-stain and with dilute carbol fuchsin for several hours.

After 12-14 hours the cultures containing the sulfapyridine showed a decreasing number of cultivable pneumococci, but no dissociation was noted. The lag phase of both cultures was negligible; the logarithmic phases of the cultures progressed nearly in parallel up to 14 hours when it continued in the case of the control culture for several hours but ceased in the case of the sulfapyridine culture. The phase of decline was somewhat accelerated in the case of the culture containing the drug.

Gram's stains showed typical Gram-positive diplococci.

Capsules were found consistently in both cultures, and no morphological or somatic changes could be detected in the organisms stained with weak carbol fuchsin.

Conclusions. 1. Sulfapyridine did not affect the capsule of the pneumococcus either *in vivo* or *in vitro* in such a way as to be ascertainable by capsule-staining or by affecting the "quellung" reaction in the presence of immune serum. 2. *In vivo* and *in vitro* experiments using sulfapyridine in pneumococcal infections or pneumococcal cultures containing leucocytes did not indicate an increase in phagocytosis of the diplococci in the presence of the drug. 3. *In vitro* there was only slight bacteriostatic effect of 10 mg % sulfapyridine in broth cultures of pneumococcus Type I until the culture neared the close of the logarithmic phase of growth. At that time a bactericidal effect was apparent along with increased bacteriostasis and the length of the phase of decline was decreased markedly. 4. *In vivo* a similar effect was noted. In mice treated with sulfapyridine there was a short period of increase in the number of organisms in the peritoneal exudate followed by a rapid decrease in the number of bacteria found until, after 18 hours, only a few organisms remained. In several days, after stopping administration

of the drug, the treated mice died and pneumococci were recovered from the heart-blood and peritoneal exudate.

5. Complete bactericidal action was not demonstrated.

6. It would seem that sulfapyridine may owe its beneficial effect in pneumococcal infections to its bacteriostatic action which may permit antibodies to be formed by the body or to be acquired passively, or allow the inhibited organisms to be disposed of by phagocytosis at the normal rate.

10703 P

Conversion of Methionine to Cystine: Experiments with Radioactive Sulfur (S^{35}).*

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A considerable amount of evidence pointing to the idea that methionine, when fed, may be converted into cystine in the animal organism has been brought forth.¹ Further evidence in support of this concept is afforded by the present experiments in which it is shown that cystine containing the radioactive sulfur isotope (S^{35}) was isolated from rats that were fed methionine containing S^{35} .

The methionine was synthesized from sulfur that contained S^{35} by a modification of the procedure described by Patterson and du Vigneaud.² † It was administered to young rats that were maintained on a low cystine diet. Animals 20 and 24 received 0.15% of the S^{35} -containing methionine daily in their diet, while rat 22 was given daily 15 mg of the same methionine subcutaneously. Rats 32, 34, and 37, maintained on the same basic diet, were fed 0.15% of ordinary methionine daily plus 0.10% of sodium sulfate that contained

* Aided by a grant from Eli Lilly and Company and the Research Board of the University of California. Technical assistance was furnished by the Works Progress Administration.

¹ Womack, M., Kammerer, K. S., and Rose, W. C., *J. Biol. Chem.*, 1937, **121**, 403; Brand, E., Cahill, G. F., and Harris, M. M., *J. Biol. Chem.*, 1935, **109**, 69; Dawbarn, M. C., *Austr. J. Exp. Biol. Med. Sci.*, 1938, **16**, 159; Beach, E. F., and White, A., *J. Biol. Chem.*, 1939, **127**, 87.

² Patterson, W. I., and du Vigneaud, V., *J. Biol. Chem.*, 1935, **111**, 393.

† The radioactive sulfur was kindly furnished by the Radiation Laboratory of the University.

radioactive sulfur. The radioactivity of the sodium sulfate was about $\frac{2}{3}$ of that of the methionine.

After a period of 5 weeks, cystine was isolated from the hair, skin, or the whole carcass of the animals by hydrolysis of the protein, precipitation of the cystine as the cuprous mercaptide, and purification by reprecipitation. The amino acid was finally obtained in pure crystalline form. It was then converted into barium sulfate.

The radioactivities of the barium sulfate samples were kindly measured by Dr. W. F. Libbey with the aid of the screen wall Geiger counter.

The cystine isolated from the hair of rats 20 and 22, the skin of rat 20, and the whole carcass of rat 24 contained radioactive sulfur. The radioactivity of the cystine isolated from the whole carcasses of rats 32, 34, and 37 was zero within the error of measurement.

The data show that some of the S^{35} contained in the methionine appeared in the protein cystine of the experimental animals. None of the radioactive sulfur contained in the sodium sulfate was found in the form of cystine.

The use of the sulfur isotope, S^{35} , affords a convenient method of studying sulfur metabolism in animals. Further work in this field is in progress.

10704 P

Effect of Choleic Acid of Vitamin K on Prothrombin Levels of Bile Fistula Rats.*

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Abundant evidence¹ is now available that administration of vitamin K to vitamin K-deficient chicks and to bile fistula animals leads to an increase in the prothrombin level and a decreased clotting time of the blood. Administration of deoxycholic acid is necessary to insure absorption of the antihemorrhagic factor when it is given orally to bile fistula animals.

It was suggested by one of us¹ that, since vitamin K combines with

* Aided by a grant from the Christine Breon Fund of the University of California. Technical assistance was provided by the Works Progress Administration.

¹ Schmidt, C. L. A., *Pac. Coast Med.*, 1938, **5**, 7.

TABLE I.

Rat No.	Days after operation	Dose of choleic acid mg per 100 g	Blood sample taken after administration of choleic acid hr	Prothrombin values before and after administration of choleic acid
1	21	10	2.5	10-50
2	29	20*	4 †	20-50
3	13	20*	4 †	25-50
4	38	20	6.5	25-39
			24	30
5	16	10	3	17-20

* Two 10 mg doses with 24-hour interval.

† Hours after last dose was administered.

deoxycholic acid, possibly the best method of administering this vitamin orally would be in the form of the choleic acid. This compound has recently been prepared by Almquist and Klose.² They showed that oral administration of the choleic acid to vitamin K-deficient chicks leads to a decrease in the clotting time of the blood. The present experiments indicate that oral administration of this choleic acid to bile fistula rats is followed by an increase in the prothrombin level of the blood.

The choleic acid was kindly supplied to us by Dr. Almquist. It was fed in varying levels to choledochocolonostomized rats. The data are given in Table I.

Although the oral administration of the choleic acid of vitamin K led to an increase in the prothrombin value of all of the bile fistula rats, the prothrombin values are below normal. It may be necessary to give larger doses in order to increase the prothrombin levels further. Experiments to determine the optimum dose of the choleic acid are in progress.

² Almquist, H. J., and Klose, A. A., *J. Am. Chem. Soc.*, 1939, **61**, 745.

Permeability of Red Corpuscles of the Dog to Sodium Ion.*

WALDO E. COHN AND ELMA TUFTS COHN. (Introduced by D. M. Greenberg.)

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The question of the permeability of mammalian erythrocytes to cations (with the exception of hydrogen ion) has long been a controversial one. The literature in the field has been reviewed by Ponder¹ and Jacobs.² Ponder states that, although there has been some evidence since 1891³ that the red blood cell is permeable to cations, it has generally been assumed that it is not.⁴ Such an assumption requires awkward corrections to explain the experimental data. To avoid postulating a cation shift across the red cell membrane, Ponder¹ states that Van Slyke and Cullen,⁵ Mellanby and Wood,⁶ and Doisy and Eaton⁷ have preferred to introduce corrections involving changes in red cell volume. However, Van Slyke and Cullen⁵ admit the possibility of a transfer of cations between plasma and corpuscles. For these volume changes, which are ascribed to water shifts, there is no good evidence, the most careful work giving results varying by as much as 100%⁸ or even varying in direction.⁹ Much of the interpretation is based on hematocrit values which are admittedly unreliable. If there is no water shift, or if it is not of the right magnitude, the results of the investigators mentioned above support the idea of a red blood cell permeable to cations.

Jacobs,² on the basis of Donnan ratios, relation of cell volume to osmotic pressure, and volume changes in solutions of varying pH

* Aided by grants from the Division of Natural Sciences of the Rockefeller Foundation and from the Christine Breon Fund of the University of California Medical School.

¹ Ponder, E., *The Mammalian Red Cell and the Properties of Hemolytic Systems*, Berlin, 1934, pp. 61, 105-108, 109-114, 128.

² Jacobs, M. H., *Ergeb. d. Biol.*, 1931, **7**, 1.

³ Hamburger, H. J., *Z. f. Biol.*, 1891, **28**, 405; Hamburger, H. J., and Bubanović, F., *Arch. int. Physiol.*, 1910-11, **10**, 1.

⁴ Ege, R., *Biochem. Z.*, 1922, **130**, 99; Koeppe, H., *Pflüger's Arch.*, 1897, **67**, 189.

⁵ Van Slyke, D. D., and Cullen, G. E., *J. Biol. Chem.*, 1917, **30**, 289.

⁶ Mellanby, J., and Wood, C. C., *J. Physiol.*, 1923, **57**, 113.

⁷ Doisy, E. A., and Eaton, E. P., *J. Biol. Chem.*, 1921, **47**, 377.

⁸ Van Slyke, D. D., Wu, H., and McLean, F. C., *J. Biol. Chem.*, 1923, **56**, 765.

⁹ Warburg, E. J., *Biochem. J.*, 1922, **16**, 153.

(with CO_2), concludes that the red blood cell is impermeable to cations. As Ponder¹ has pointed out, the evidence is inconclusive on all 3 points, and Jacobs himself admits the possibility of alternate explanations.

That a so often investigated subject should still be in such a controversial state is due principally to the indirectness of the previously available methods of study. The most direct experiments were performed by adding salts to blood *in vitro* and measuring the changes in cation concentration and in volume of whole blood, plasma and cells.¹⁰ The time for any permeation to occur was thus limited to a relatively few minutes and the results depended upon the analysis of very small differences in the cation content and volume of the cells. The more indirect methods and experiments with cells in saline or under other abnormal conditions are subject to criticism.¹

The discovery of the artificial radioactive isotopes of sodium and potassium has opened up an entirely new method of approach, making it possible to study the problem *in vivo* by simple, direct methods.¹¹ The artificial radioactive isotope of sodium (Na^{24}) has the same chemical behavior as the naturally occurring one (Na^{23}), yet may be detected and measured by its decomposition in which it emits electrons and gamma radiation.

We are investigating the subject of the permeability of mammalian erythrocytes to cations with this method and report herewith the results of experiments with radioactive sodium in the dog.

Radioactive sodium,† in 10-25 ml isotonic saline, was injected intravenously into normal dogs. At various periods of time (captioned "*in vivo*" in the table), blood samples were drawn into heparin and centrifuged after varying periods of time ("*in vitro*" in the table) for 10 minutes at about 2400 r.p.m. Except where it is stated that the sample was drawn under oil, no precautions were taken against loss of CO_2 . After centrifugation, the plasma was withdrawn as completely as possible with a pipette and the cells were mixed with a large volume of isotonic sucrose (equal to 1.12% NaCl) and centrifuged. This was repeated twice to remove all adherent plasma. No evidence of loss of sodium from the cells was

¹⁰ Eisenman, A. J., Mackenzie, L. B., and Peters, J. P., *J. Biol. Chem.*, 1936, **116**, 33; Wakeman, A. M., Eisenman, A. J., and Peters, J. P., *J. Biol. Chem.*, 1927, **73**, 567.

¹¹ Hevesy, G., *Enzymologia*, 1938, **5**, 138; Lawrence, J. H., *Handbook of Physical Therapy*, Am. Med. Assn., 1938.

† We are indebted to Professor E. O. Lawrence and the staff of the Radiation Laboratory of the University of California for supplying us with the radioactive sodium used in these experiments.

TABLE I.

Exp.	Sample	Time (minutes)			Log t	Hematocrit (H)	Na ²⁴ /ml Plasma		× 100
		In Vivo	In Vitro	Total			Na ²⁴ /ml Plasma		
4	1A	7	8	15	1.176	44	9.4		
	2	32	13	45	1.653	44	12.6		
	1B	7	143	150	2.176	51	25		
5	1A	7	3	10	1.000	47	5.5		
	2A	27	4	31	1.491	51	10		
	3A	45	2	47	1.672	51	11		
	4	397	3	400	2.602	47	44		
	5	1530	5	1535	3.186	47	65		
	1B	7	94	101	2.004	45	22.4		
	2B	27	74	101	2.004	48	23		
	3B	45	56	101	2.004	52	20		
	1	5	6	11	1.041	54	4.5		
	2	5	32	37	1.568	53	7.8		
8	3	5	61	66	1.820	54	14		
	4	5	182	187	2.272	53	32		
	1A	5	15 (oil)	20	1.301	52	4.8		
	1B	5	134 (oil)	139	2.143	52	23		
11	1C	5	279 (oil)	284	2.453	51	32		
	2	135	4	139	2.143	51	20		
	3	280	4	284	2.453	50	27		
	1	840	10	850	2.929	56	56		
13	2	1020	10	1030	3.013	56	56		

Time *in vivo* = time between injection of Na²⁴ and withdrawal of blood sample.Time *in vitro* = time between withdrawal and centrifugation.

noted, and in several cases measurements of the wash solutions and hematocrits showed a Na^{24} content equal to the plasma left behind.

The relative amount of Na^{24} in plasma was measured directly, after evaporating an aliquot in a 10 ml Coors ashing capsule, by means of a Lauritzen electroscope. The relative amounts of Na^{24} in whole blood and cells were measured on trichloroacetic filtrates. Corrections were made for the volume of the protein precipitate in each case. The radioactivity of each sample is expressed in arbitrary units and is directly proportional to the Na^{24} content.

The data and results are shown in Table I. The hematocrit values were calculated from the Na^{24} content of whole blood, plasma, and cells and agreed with those found (from graduated centrifuge tubes) within 5 volume %.

In Figure 1 the ratios of red corpuscle Na^{24} to plasma Na^{24} have been plotted against the logarithm of the total time which elapsed

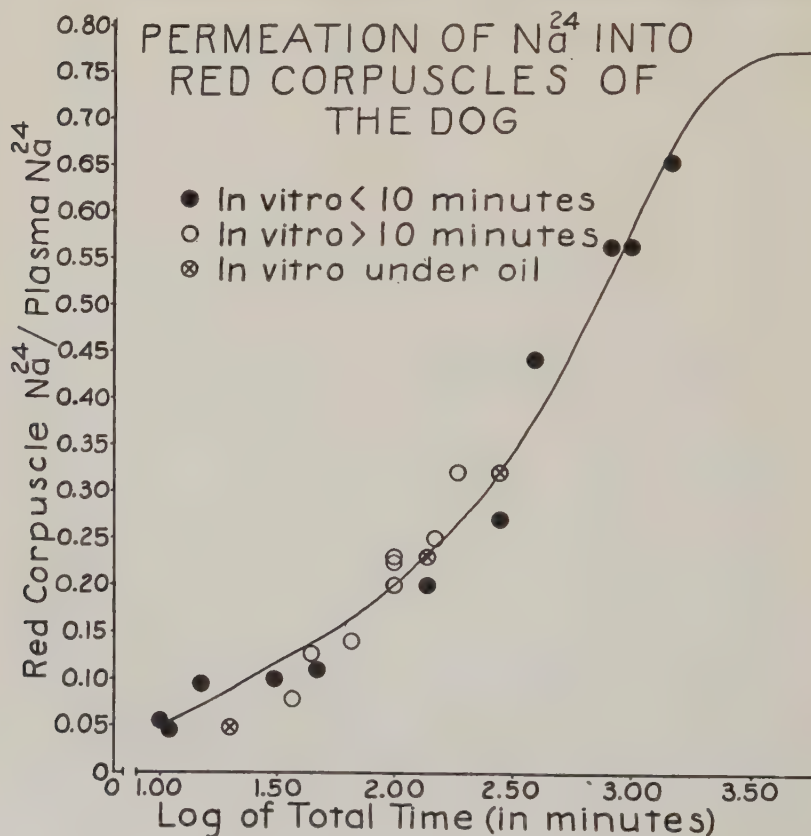


FIG. 1.

from the injection of Na^{24} to the centrifugation of the blood samples. The points represent the experimental data. The solid curve has been calculated by assuming that a simple equilibrium exists between corpuscle and plasma sodium, and that the rate at which Na^{24} enters the corpuscle from the plasma is proportional to the difference in their respective concentrations, allowing for the normal difference in sodium content.

The following conclusions may be drawn from these results: first, that the red corpuscles of the dog are permeable to sodium ion; second, that the rate of permeation by sodium ion is nearly the same with blood *in vitro* as *in vivo*; third, that the permeation appears to be of a simple equilibrium nature.

We wish to express our gratitude to Professor D. M. Greenberg for his advice and assistance in these experiments, and to Mr. A. J. Glazko for technical aid.

10706

Blood Sugar in Cats with Diabetes Insipidus Before and After Adrenalectomy.

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Cats with experimental diabetes insipidus produced by interruption of the nervous connections of the posterior pituitary show rather unusual effects after bilateral adrenalectomy.^{1, 2, 3} Whereas ordinary adrenalectomized cats show striking diminution in the serum sodium and chloride levels, adrenalectomized d.i. cats have normal serum concentrations of these substances even when *in extremis*. There is a more or less marked elevation of blood potassium, however, similar to that occurring in ordinary animals. The survival time after adrenalectomy is reduced from an average of 8 days for ordinary cats to an average of 3 for d.i. cats. A negative water balance is set up in both cases, the d.i. cats losing water the more rapidly. The total loss of water is not a lethal one; however, it has been suggested that the rapid external loss plus shifts in fluid within

¹ Winter, C. A., Gross, E. G., and Ingram, W. R., *J. Exp. Med.*, 1938, **67**, 251.

² Ingram, W. R., Winter, C. A., and Gross, E. G., *Am. J. Physiol.*, 1938, **122**, 143.

³ Winter, C. A., Ingram, W. R., and Gross, E. G., 1939, in press.

the body may account for the short survival time of the d.i. cats. Another factor must also be considered. In the authors' experience (*cf.* also Ingram and Barris⁴) d.i. cats may show signs of altered carbohydrate metabolism as indicated by increased sensitivity to insulin. In the absence of other apparent causes this change may be provisionally accounted for by some degree of suppression of certain anterior lobe functions. Total hypophysectomy is said to shorten the survival time of adrenalectomized rats.⁵ A breakdown in carbohydrate metabolism (diminution in blood sugar and carbohydrate reserves) has been associated with death in adrenal insufficiency by Britton and Silvette;⁶ this association is denied by Parkins, Hays and Swingle⁷ and others, and Buell, Anderson and Strauss⁸ report that such changes in carbohydrate metabolism do not necessarily cause rapid death in rats receiving high salt diets. Corey and Britton⁹ observed that adrenalectomy following hypophysectomy resulted in pronounced diminutions in blood sugar and muscle and liver glycogen levels. The participation of the adrenal cortex in certain phases of the metabolism of carbohydrate cannot be denied in view of the work of Long and his coworkers.¹⁰ That loss of this participation is the cause of death in adrenal insufficiency has not, however, been generally accepted. Nevertheless, it seemed necessary to determine if suppression of the anterior lobe as manifest by hypersensitivity to insulin could be associated with the short survival times of d.i. adrenalectomized cats. Attempts to do this are herewith reported along with observations on the terminal blood sugar levels in ordinary and d.i. adrenalectomized cats.

Pre-adrenalectomy insulin sensitivities in a group of 10 d.i. cats were determined by plotting blood sugar curves following intravenous administration of 0.17 unit of insulin per kg.⁴ These cats were later adrenalectomized and the blood sugar concentration determined again just before death. Six of these animals were on pitressin treatment during the post-adrenalectomy period.* The results are tabulated in Table I.

⁴ Ingram, W. R., and Barris, R. W., *Am. J. Physiol.*, 1936, **114**, 562.

⁵ Samuels, L. T., Schott, H. F., and Ball, H. A., *Am. J. Physiol.*, 1937, **120**, 649.

⁶ Britton, S. W., and Silvette, H., *Am. J. Physiol.*, 1932, **100**, 701.

⁷ Parkins, W. M., Hays, H. W., and Swingle, W. W., *Am. J. Physiol.*, 1936, **117**, 13.

⁸ Buell, M. V., Anderson, I. A., and Strauss, M. B., *Am. J. Physiol.*, 1936, **116**, 274.

⁹ Corey, E. L., and Britton, S. W., *Am. J. Physiol.*, 1937, **118**, 15.

¹⁰ Long, C. N. H., and White, A., *Ergebn. d. Physiol.*, 1938, **40**, 164.

* Thanks are due Dr. Oliver Kamim of Parke, Davis & Co. for generous supplies of Pitressin.

TABLE I.
Effect of Adrenalectomy on Blood Sugar of Normal Cats and Cats with Diabetes Insipidus.

Cat	Pre-adrect. Urine Vol. cc	Initial Blood Sugar mg%	Terminal Blood Sugar mg%	Days Survived	Insulin Sensitivity
Cats with Polyuria—Adrenalectomy.					
32	416	82	82	2*	N
39	206	70	70	7	N
49	202	71	46	4	H++++
54	328	77	88	2	H+
26	423	85	24	2*	?
23	232	92	44	4*	?
Cats with Polyuria—Adrenalectomy, Pitressin Treatment.					
47	490	77	67	6	N
51	536	61	96	7	N
59	396	66	84	4	N
40	557	98	86	5	H+
55	715	80	61	4	H-+
58	567	69	55	2	H-+++
57	687	—	94	7	?
Cats without Polyuria.					
31	119	65	25	6	
29	112	65	54	9	
43	121	79	66	5	
60	142	—	88	5	Pitressin
Na5	101	75	59	5	"
Na7	117	—	62	6	"

* After discontinuing cortin treatment.

Normal sensitivity to insulin indicated by N.

Hypersensitivity to insulin indicated by H.

It will be noted that 5 of 10 d.i. cats showed hypersensitivity to insulin in varying degrees. Cat 32, of normal pre-adrenalectomy sensitivity, had as short a survival time as the hypersensitive cats (49 and 54). On pitressin treatment the survival times of the normally sensitive cats were slightly greater than those of the hypersensitive animals. Two d.i. cats (32 and 39) of previously normal sensitivity showed terminal blood sugars of 70 and 82 mg %. Two d.i. cats (49 and 54) hypersensitive to insulin had terminal blood sugars of 46 and 88 mg %, and in the latter instance (Cat 54) the survival time was the shortest. D.i. cats on pitressin treatment show but slightly higher terminal blood sugars. In general, of 13 d.i. adrenalectomized cats, only 3 showed terminal blood sugars below 50 mg %, only one of which (cat 26, of unknown insulin sensitivity) was at a convulsive level. Of 6 non-polyuric adrenalectomized cats only one showed a strikingly low blood sugar.

These data advance no evidence that the short survival times of d.i. adrenalectomized cats are primarily associated with anterior lobe suppression as indicated by insulin sensitivity. Furthermore, there seems to be no justification for suggesting that death in these or in

ordinary adrenalectomized cats is necessarily related to low levels of blood sugar. In the authors' experience blood sugar levels of 50-70 mg % in cats are not incompatible with life, while the convulsive level lies between 20 and 28 mg %. No statement as to the carbohydrate reserves of these animals can be made. It is not unreasonable to presume that these are reduced, especially in view of the period of anorexia preceding death from adrenal insufficiency and the defect in conversion of protein to carbohydrate which follows loss of the adrenal cortex.¹⁰ With the exception of the 2 cases mentioned, however, the blood sugars were not at a lethal level. It has recently been observed, moreover, that d.i. adrenalectomized cats may be maintained for considerable periods of time on high intakes of sodium and chloride, with maintenance of relatively dilute blood, in the absence of cortin (Ingram, Winter and Gross¹¹). This would lend further weight to the suggestion that the short survival periods and death of d.i. adrenalectomized cats are in general not associated with suppression of the anterior lobe nor with decline in the blood sugar level due to anterior lobe involvement or loss of the adrenal cortex. The cause of the early demise of these animals may more likely be connected with shifts of fluid shown to take place within the body by other workers, together with rapid loss of water from the body in the absence of the fluid-conserving influence of the normally innervated posterior lobe.

Summary. Cats with posterior pituitary inactivation may die in adrenal insufficiency with blood sugars in the normal range, even if these animals have previously been found to be hypersensitive to small doses of insulin. This is true whether or not the animals are under treatment with pitressin. There is no evidence that the short survival period of untreated diabetes insipidus cats after adrenalectomy is associated with the blood sugar level or partial anterior lobe suppression.

¹¹ Ingram, W. R., Winter, C. A., and Gross, E. G., *Proc. Am. Physiol. Soc.*, 1939, p. 128.

10707

Effect of Wheat Germ Oil Upon E-Deficient Muscular Dystrophy.

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College of Medicine.*

It has been shown that 5-months-old rats reared from birth on a vitamin E-deficient diet show dystrophic changes in the skeletal muscle.¹ This report is concerned with attempts to promote recovery in such animals by the administration of wheat germ oil or its vitamin E-containing concentrate.

E-deficient rats were prepared by placing prospective mothers on an E-deficient diet (Olcott²) when about 18 days pregnant. The mothers were continued on this diet throughout the suckling period. The young rats were weaned at 22 days of age and continued on the E-deficient diet to 5 months of age. At this time some were used for study, and the remainder divided into 3 groups as follows: (1) continued on the E-deficient diet, (2) E-deficient diet plus 10% of wheat germ oil, (3) E-deficient diet plus a weekly dose of 30 mg of a concentrate of wheat germ oil. Thirty mg of this concentrate was proven adequate to carry an established E-deficient female through the gestation and suckling period. A part of the animals of each of these 3 groups were studied at an age of 7 months, the remainder at an age of 8 months.

The gastrocnemii of the above animals were studied with regard to histologic changes, water and chloride concentration and maximum power. The maximum power was taken as the tension developed against an isometric lever when the intact muscle was stimulated with condensor discharge shocks at the rate of 50 per second. This same muscle was then removed from the animal, weighed and fixed in Zenker-formol. Sections were stained with hematoxylin and eosin.

The male rats were apparently more susceptible to the E-deficient regime than were the females. Therefore, the average results for the 2 sexes are shown separately (Tables I and II). Average values from normal rats reared on a stock diet are included for comparison.

Morphologically, the changes observed in the untreated E-deficient

¹ Knowlton, G. C., and Hines, H. M., *PROC. SOC. EXP. BIOL. AND MED.*, 1938, **38**, 665.

² Olcott, H. S., *J. Nutrition*, 1938, **15**, 221.

TABLE I.
Males.

Group	Normal	E-deficient diet			WGO* supplement			Concentrate† supplement		
		5	7	8	7	8		7	8	
Age, mo	6									
Muscle tension in g/g	1763	1177	1168	1106	1166	995		1149	1112	
No. of animals	12	4	2	2	3	3		2	4	
Water, %	75.61	75.87	76.11	76.48	75.82	75.83		75.54	76.11	
Cl, mg/100 g muscle	45.5	53.1	53.1	66.7	48.5	48.3		51.6	47.4	

TABLE II.
Females.

Group	Normal	E-deficient diet			WGO* supplement			Concentrate† supplement		
		5	7	8	7	8		7	8	
Age, mo	6									
Muscle tension in g/g	1842	1489	1397	1339	1426	1269		1379	1297	
No. of animals	13	4	3	3	4	2		2	4	
Water, %	75.51	75.56	75.81	75.98	75.08	75.19		75.42	75.53	
Cl, mg/100 g muscle	46.1	49.8	45.2	53.7	44.8	45.2		46.7	46.2	

* Wheat germ oil.

† Concentrate of wheat germ oil.

rats were similar to those reported by Evans, Emerson and Telford,³ but are less extensive than those observed by Olcott,² Pappenheimer⁴ and Goettsch and Ritzmann⁵ in suckling rats of E-deficient mothers. In our series lesions were fully developed when the rats were first examined at the age of 5 months, and there was little, if any, change in the histological picture during the following 3 months. In no case were degenerative lesions present in more than a few fibers in any one section. The degenerative changes consisted of hyaline necrosis involving the entire fiber or merely a segment of the latter. The necrotic zone was usually infiltrated with large mononuclear cells and occasionally with a few neutrophilic and eosinophilic polymorphonuclear leukocytes. In some of the affected areas the necrotic tissue had been resorbed and the fibers were collapsed, but the sarcolemma was still distinct and numbers of large mononuclears and proliferating muscle nuclei were present. No inflammation of the interstitial tissues was seen, as in the more severe lesions described by Olcott² and by Pappenheimer.⁴

Along with these degenerative changes were to be seen evidences of regeneration of two types:

(1) An "early" form in which the muscle fibers were small, with a basophilic sarcoplasm, and with numerous nuclei, commonly centrally placed within the fiber. The nuclei were oval and vesicular, which implied active proliferation. Few myofibrils were seen in these fibers.

(2) A type in which the fibers were of nearly normal size with both longitudinal and cross striations, and with more slender nuclei. The nuclei occurred singly, in small groups, or in chains of 6 to 8, and were situated both in the subsarcolemmal region and centrally in the fiber. These changes were interpreted as representing "late" stages of regeneration. Neither in this group nor in the treated group described below was there any replacement fibrosis or adiposity within the muscles.

All treated animals, with one exception, showed complete absence of necrosis, and even this one case showed merely one necrotic fiber in 2 sections. In 4 other cases an occasional collapsed fiber was seen. It is possible that in these particular cases the original necrosis occurred before the supplement was added to the diet. In no case were regenerative lesions of the "early" form described above to be

³ Evans, H. M., Emerson, G. A., and Telford, I. R., *PROC. SOC. EXP. BIOL. AND MED.*, 1938, **38**, 625.

⁴ Pappenheimer, A. M., *Am. J. Path.*, 1939, **15**, 179.

⁵ Goettsch, M., and Ritzmann, J., *J. Nutrition*, 1939, **17**, 371.

found. Regenerative lesions of the "late" type were fairly common, approximately as common as in the untreated animals.

It is apparent from data given in the tables that the chloride and water concentrations, which were elevated in the E-deficient animals, returned to the normal range after treating the rats for 2 to 3 months with wheat germ oil or with vitamin E concentrate.

In marked contrast, the muscular power was definitely impaired by the E-deficient diet. This loss of power, as the tables show, was approximately 30%, and yet it was not so great as to handicap the animal in its normal cage activity. The muscular power, however, showed no improvement after feeding the vitamin supplements. This is of particular interest in view of the fact that muscles of the treated animals had normal concentrations of chloride and water, and showed no necrosis or "early" regenerative lesions. It is possible that all of the muscle fibers were weak, or that only those fibers showing "late" regeneration were weak. Evidently the physiological damage is something rather subtle which cannot easily be evaluated by histological or chemical methods.

Summary. Muscular dystrophy was produced in rats by feeding a diet deficient in vitamin E, beginning at birth. At the age of 5 months muscle necrosis and muscle regeneration was evident histologically and chemical studies showed elevation of the chloride and water concentrations of the muscles.

Subsequent addition of wheat germ oil or vitamin E concentrate to the diet for 2 to 3 months resulted in complete recovery with respect to the concentration of chloride and water in the muscle. Histologically the muscles showed practically no evidence of necrosis. Despite the chemical and histological evidence of recovery the muscles still lacked the ability to develop the normal amount of tension when tested by quantitative methods.

10708 P

Sulfanilamide and Sulfapyridine in Type III Lobar Pneumonia of Rats.

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In testing experimentally the relative therapeutic effects of sulfanilamide and sulfapyridine in Type III pneumococcic pneumonia we have used the same method that was used successfully in previous experiments with Type I infections in rats.¹ The culture used was recently isolated from the lung of a fatal case of lobar pneumonia in a man. The culture was maintained on blood agar and passed through rats at least once a week in order to keep its virulence at a maximum. Eighteen-hour bouillon cultures diluted to 10^{-6} and suspended in mucin have killed rats when introduced into the lung by intrabronchial insufflation in doses of 0.1 cc. Death resulted within 5 days from lobar pneumonia, empyema and pericarditis, characterized by a profuse gelatinous exudate.

Therapy was begun about 4 hours after the time of injection of the infecting dose and consisted of the administration by stomach tube of sulfanilamide or sulfapyridine,* suspended in 5% mucin. The initial dose in each case was 250 mg and the daily maintenance dose was 125 mg, continued for 6 days in the first group and for 10 days in the second group of rats. No claim is made that optimal results are obtained by daily administration of the drugs but it is sufficiently effective for comparative results and involves less frequent handling of the sick animals, which in itself probably influences favorably the number of survivors.

Of the first group of 37 rats, weighing on the average about 185 g, 12 were used as untreated controls. All of these died from infection within 4 days. Of the 12 receiving sulfanilamide, 2 survived indefinitely, the others dying at intervals up to 14 days. Of the 13 rats receiving sulfapyridine, 5 lived indefinitely and the others died at various intervals up to 24 days after inoculation.

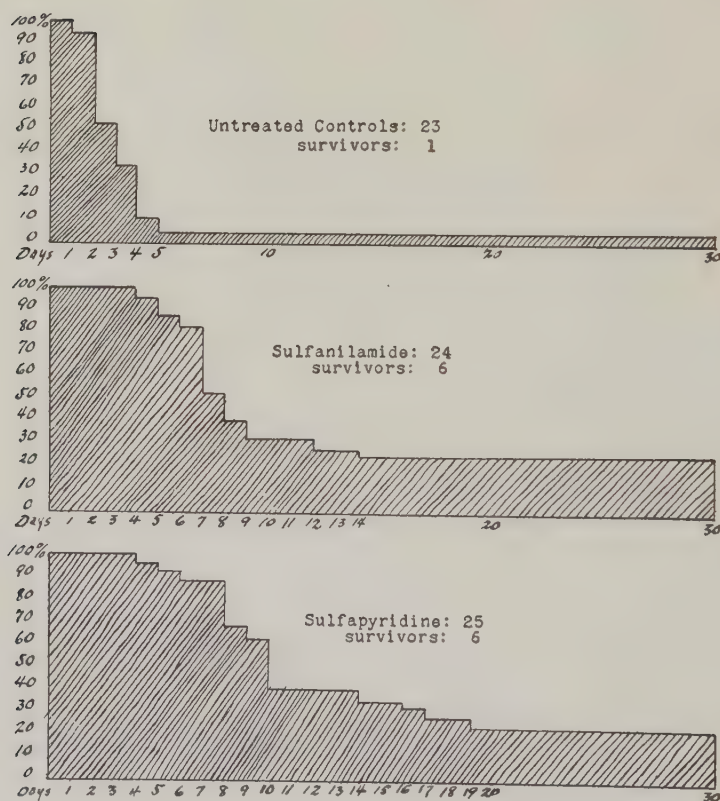
In an attempt to ascertain whether or not the survival rate could be improved by prolonging the period of treatment, a second group of 35 rats was infected in the same manner and by the same dose that was used in the first group. The treatment also was the same with

¹ Kepl, M., and Gunn, F. D., *PROC. SOC. EXP. BIOL. AND MED.*, 1939, **40**, 529.

* Donated by Merck and Company, Rahway, N. J.

the exception that the daily maintenance dose was continued for 10 days instead of 6 days. One of the 11 untreated controls survived and was sacrificed on the 20th day. There was no evidence of persistent infection but a black spot (India ink tracer) was found in the base of the left lung, indicating that the lung had been properly injected. The others died from infection within 5 days, all except 2 showing lobar consolidation of the lungs. These 2 showed empyema and evidence of septicemia. Of the 12 rats receiving sulfanilamide, 4 survived and 8 died in 7 to 9 days. Of the 12 receiving sulfapyridine, only one survived indefinitely, the rest dying in 5 to 19 days with lesions differing from those of the controls in that they showed

Sulfanilamide and Sulfapyridine in Type III Pneumonia.



Infecting dose: 0.5 cc. of 10^{-6} dilution; 18 hour bouillon culture.

Number of animals surviving at various intervals expressed in %.

FIG. 1.

fewer instances of purulent pleuritis and pericarditis. Eight, however, showed fully developed lobar pneumonia and the others evidence of septicemia (splenitis, pyemic abscesses, etc.) at the time of death.

Since the number of survivors was not increased in significant degree by prolongation of the period of therapy (actually decreased in the case of sulfapyridine) we have combined the figures from the 2 groups for comparative purposes and they are illustrated in percentage values in the block graph (Fig. 1).

Summary. When treatment was begun 4 hours after the time of inoculation with the infecting dose, both sulfanilamide and sulfapyridine were partly effective in protecting rats against Type III pneumococcal pneumonia. There was no significant difference under the conditions stated between the effects of the 2 drugs in preserving life but the survival time of animals dying of infection was on the average 2 days longer in the group treated with sulfapyridine. Prolongation of the period of treatment from 6 to 10 days did not appear to reduce the mortality but the number of animals used was insufficient to permit definite conclusions on this point. The complications of empyema and purulent pericarditis were less frequent in animals treated with sulfapyridine even though the period of survival was longer in the treated animals.

10709 P

Sulfapyridine in Experimental Lobar Pneumonia in the Dog.

L. A. GREGG, C. G. LOOSLI AND M. HAMBURGER, JR. (Introduced by O. H. Robertson.)

From the Department of Medicine and the Douglas Smith Foundation for Medical Research, the University of Chicago.

The reported mortality of pneumococcic pneumonia in patients treated with sulfapyridine has been remarkably low. Yet, in controlled experiments with the drug in pneumococcus-infected mice and rats, the results obtained by different investigators have not been uniformly so striking. Animals used in those studies are species highly susceptible to the pneumococcus. Man, conversely, is relatively resistant and is in that regard, resembled by the dog. O. H. Robertson and coworkers¹ have shown that lobar pneumonia

¹ Robertson, O. H., *J. Am. Med. Assn.*, 1938, **111**, 1432.

TABLE I.
Mortality and Duration of Fever in Controls and in Dogs Treated with Sulfapyridine.

Sulfapyridine		No. of dogs	Duration of Fever* Number of dogs showing drop of temperature to normal					Total Mortality
Begun at intervals after infection, hr	Total amt given, g		within 24 hr	between hours			after 96 hr	
3	5 to 7	10	0	24-48	48-72	72-96	after 96 hr	
12	5 to 6	5	0	5	3	1	1	
18-24	3.7 to 6	9	1†	3	2	—	—	0
Control dogs				2	5	1	—	
Surviving		16	1†	4	9	1	1	
Dying†		16	1	4	4	4	3	50%

Dosage of drug: initial dose 2 or 3 g, followed in 6-8 hours with $\frac{1}{2}$ g three times a day for 2 to 5 days.

* Temperatures (rectal) above 102.9°F considered to indicate fever.

† Figures in columns 4-7 indicate time of death.

‡ Temperature not taken until 24 hr.

TABLE II.
Production of Lobar Pneumonia in Dogs Receiving Sulfapyridine Prior to Infection.
Dogs sacrificed 24 hours after infection.

Dog No.	Infecting dose of culture cc	Concentration of drug in the blood		Extent of lung involvement at autopsy, lobes	Lung puncture at autopsy		Rectal temperature when sacrificed, degrees F.
		when infected mg%	when sacrificed mg%		Gram-pos. diplococci	Neufeld reaction	
251 T	1.0	10.0	2.8	>1	+	+	104.0
281 T	1.0	4.9	3.7	>1	+	+	104.0
271 T	0.02*	3.3	3.6	1 $\frac{1}{3}$	0†	—	103.8
272 T	0.02*	18.0	2.8	1	0	—	102.5
273 T	0.02*	12.0	5.1	<1	+	+	104.3
280 T	0.02*	12.8	5.0	>1	+	+	102.4

* Suspended in 1 cc of starch-broth medium.

† Pneumococci present on culture.

> = more than.

< = less than.

Quantity of drug before infection: 3 to 5 g; after infection: 2 to 4 g.

can be produced in the dog, which is in all essential respects comparable to the human disease.

In the present investigation dogs weighing 8 to 15 kg were infected intrabronchially by the method of Robertson and Fox² with 1 cc of Type I pneumococcus culture followed by 3 cc of mucin, resulting in a disease 50% fatal in the controls. Sulfapyridine* was given orally in capsules or compressed tablets.

Of 24 dogs receiving the first dose of drug 3 to 24 hours after infection, none died (Table I). In 9 instances where serial blood-determinations were made, the content of free sulfapyridine was generally 2 to 6 mg %.

In the group not treated until 18 or 24 hours after infection, one had a bacteremia of 26 colonies per cc of blood at the time of treatment and another had 230. Of the 5 control dogs whose blood at 24 hours contained on culture more than 20 colonies per cc, none survived.

Four additional bacteremic dogs, all but one having a colony count of more than 1,000 per cc of blood were selected for treatment. A total of 5.7 or 6 g of sulfapyridine was given to each, beginning 29 to 72 hours after infection. All died, one with empyema and another with purulent pericarditis. In the other 2, the blood culture became sterile within 48 hours, but several days later severe anemia and jaundice appeared. Post-mortem cultures from the latter 2 yielded no pneumococci. Profound anemia with jaundice has been observed in this laboratory otherwise but once in over 1,000 dogs with experimental pneumonia.

Table II shows that with the method here employed for its production, a lobar pneumonia can evolve despite the administration of large doses of sulfapyridine before and after infection, and despite its presence in the blood in concentrations equal to or greater than those obtaining under conditions when the drug is regularly curative.

² Robertson, O. H., and Fox, J. P., *J. Exp. Med.*, 1938, **69**, 229.

* We are indebted to Merck and Company for supplying us with sulfapyridine.

10710 P

Concentration of Sulfanilamide in Human Bile.

R. BETTMAN AND E. SPIER. (Introduced by H. Necheles.)

From the Departments of Gastro-Intestinal Research, the Gallbladder Study Group, and the Department of Chemistry of Michael Reese Hospital, Chicago.

In view of the difficulty in introducing effective bactericidal drugs into the gallbladder we studied this problem by examining human gallbladder bile for its contents of sulfanilamide, following oral administration of this drug. For this purpose in 11 patients who were about to be operated upon for gallstones, sulfanilamide was given before operation and specimens of the bile and blood taken at operation in cases of cholecystectomy. Sulfanilamide was administered in individual doses of 15 grains, with the addition of sodium bicarbonate. The sulfanilamide content of blood and bile were determined by Marshall's method for blood and a slight modification of this method for bile.¹ In those patients in whom the common duct was drained common duct bile was taken.

We found in every case that the sulfanilamide could be detected in the bile. In 3 out of 4 cases (Nos. 1-4) in which the Graham Cole test had shown good concentration of the dye in the gallbladder before operation, the concentration of sulfanilamide in the gallbladder bile was higher than in the blood, and in 2 out of 3 cases (Nos. 5-7) in which the Graham Cole test had indicated poor concentration, the sulfanilamide concentration in the bile was lower than in the blood. In the 4 cases in which common duct bile was used the results are variable (Nos. 8-11). In patient No. 10 repeated determinations of sulfanilamide in liver bile (common duct drainage) and blood showed that below a concentration of 4.2 mg % of the drug in the blood, none appeared in the bile.

Summary. This series of 11 cases shows us that sulfanilamide is excreted in the bile and that it may be concentrated considerably in the gallbladder. Further work is being done to see whether or not on account of the concentration of sulfanilamide in the gallbladder this drug might be of therapeutic value in the case of infected gallbladder.

We want to thank Drs. H. Necheles and D. Cohn for their help.

¹ Marshall, E. K., Jr., *PROC. SOC. EXP. BIOL. AND MED.*, 1937, **36**, 422; *J. Biol. Chem.*, 1937, **122**, 267.

TABLE I.
Concentration of Sulfanilamide in Human Bile.

Case No.	Sex	Age	Graham Cole Test	Surgical and pathological findings	Sulfanilamide			
					Administered	Concentration mg%		
					No. of days	Total dose grains	In bladder bile	In duct bile
1	M	42	Good concentration. Single stone.	Cholecystitis and stone	2	90	2.5	1.3
2	F	50	Good concentration. Single stone	Cholecystitis and stone	2	90	Trace	3.1
3	F	46	Good concentration. Stones.	Cholecystitis and stones	2	105	10.1	2.3
4	F	53	Good concentration.	Single stone in gall bladder	3	135	5.8	3.7
5	F	50	Poor concentration. Single stone.	Marked cholecystitis	2	60	2.4	5.7
6	F	35	Poor, if any concentration.	Cholecystitis and stone in gallbladder and cystic duct	2	90	Trace	1.7
7	F	54	Poor concentration. Stones.	Thick inspissated bile, many stones	2	90	6.6	2.4
8	M	51		Adhesions, cholecystitis, and stones	2	90		Trace
9	M	61		Common duct stone.	1	15		+
10	F	40	No concentration.	Drainage of common duct.	1	60		+
11	F	58	" "	Common duct stone	2	100		++
				Common duct stone	2	75		5.7
				Drainage of common duct. (Old cholecystectomy.)				3.5

10711 P

Experimental Hypothalamico-Hypophyseal Obesity in the Rat.*

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From the Institute of Neurology, Northwestern University Medical School.

Smith¹ produced obesity in rats by injecting chromic acid into the hypophysis via a subtemporal approach. Because hypophysectomy did not bring about the same result, Smith reasoned that hypophyseal deficiency could not be responsible for the adiposity—and that hypothalamic damage incurred during the chromic acid operation must have been the cause. Ranson, Fisher, and Ingram² have reported the effect of hypothalamic lesions on fat deposition in a large number of cats and monkeys observed in our laboratory, and lately the guinea pig has been added to this list. In all 3 species the results have been negative.

In an effort to discover the reason for this divergence of findings we have repeated, with modifications, Smith's chromic acid injections on rats. About 0.01 cc of 5% chromic acid was injected through a needle with a curved tip into the hypophyses of albino rats via a parapharyngeal approach. Using this method we hoped to minimize the danger of simultaneous hypothalamic damage. We have also placed lesions in the hypothalami of rats with the Horsley-Clarke instrument. Two electrolytic lesions were placed on each side in the lateral hypothalamic areas between the levels of the optic chiasma rostrally and the infundibulum caudally.

All rats were operated at about the time of sexual maturity. They had litter mate controls, and were allowed to survive well over the maximum time elapsing before any obese rat showed definite signs of its disorder. They were weighed at weekly intervals. At autopsy the brains were fixed in formalin, and the hypophyses in either Zenker-formol or Champy fluid. Serial sections of the hypothalami and neighboring areas were cut and stained with cresyl violet; the hypophyses were stained by either the Haterius modification of the Masson stain, or by the Severinghaus technic.

The chromic acid injections were performed on over 50 rats, but only 3 showed any sign of obesity—2 to a marked, and one to a mild degree. All 3 were stunted in length compared to their con-

* Aided by a grant from the Rockefeller Foundation.

¹ Smith, P. E., *Am. J. Anat.*, 1930, **45**, 205.

² Ranson, S. W., Fisher, C., and Ingram, W. R., *Endocrinol.*, 1938, **23**, 175.

trols; even so, the 2 heavier animals outweighed the normal litter mates, in one case by 7%, in the other by 40%. Histological examination of the base of the brains of these 3 rats showed damage extensively distributed from the tuberculum olfactorium rostrally to the mammillary bodies caudally. All destruction was fairly superficial with respect to the base of the diencephalon, and seemed to point either to damage done directly to the hypothalamus by the chromic acid, or injury to the vascular supply. The hypophysis of the mildly obese rat showed almost complete replacement by connective tissue, with only a few deeply basophilic nests of cells scattered along the edge of the scar. Each of the 2 markedly obese rats retained about 25% of its anterior lobe. The one surviving the shorter interval displayed a lack of normal well granulated basophils and an excessive number of what appeared to be large lightly staining basophils. The other (and fattest) rat showed a large portion of its hypophyseal remnant to be filled with a branching network of deeply staining basophilic cells. These last 2 animals also exhibited a scarcity of acidophils; those present were small and stained lightly.

Of the other rats which received chromic acid injections it may be said that only 4 or 5 had any hypothalamic damage worth mentioning, and this was minor compared to the obese cases. The hypophyses of the negative cases underwent all degrees of destruction from complete to negligible. In those cases where appreciable amounts of the anterior lobe remained the cell picture seemed fairly normal.

The results of the electrolytic hypothalamic lesions are quite different. Twelve rats have been operated to date, and practically every one has exhibited a pronounced and rapidly appearing obesity. Some rats have become almost twice as heavy as their controls. Dwarfing is only sometimes evident. Only 2 of this group have been sacrificed, and no histological studies have as yet been made; but the autopsy findings give a clue to the condition to be expected in the others. There was extensive damage to the ventral surface of the hypothalamus, involving particularly the anterior half, and being laterally disposed. Simple inspection revealed no abnormality of the hypophysis.

Injury to the hypothalamus in the rat would appear to be capable of causing marked adiposity but changes in the hypophysis may be revealed on microscopic examination.

10712 P

Effect of Spermine on Tissue Oxidations.*

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(Introduced by F. C. Koch.)

From the Department of Biochemistry, University of Chicago.

Little is known of the biological rôle of spermine (N, N'-dipropylamino-diaminobutane) despite its widespread occurrence in mammalian tissues (human semen, bovine pancreas, spleen, thyroid, lung, ovary, brain, ocular tissue, muscle, liver, and intestine). We have isolated spermine from dilute acid extracts of swine duodenal mucosa. The fact that these extracts produce a hyperglycemia in rabbits led us to investigate the effects of the pure base on the blood sugar level. The intramuscular injection of 15-25 mg per kilo of spermine into fasted, male rabbits weighing about 2 kilos, induces a definite and prolonged hyperglycemia.

In order to illuminate further the effects of spermine on the intact organism we have undertaken a general study of the action of the base on the respiration of isolated mammalian tissues. The addition of spermine to guinea-pig brain brei suspended in a phosphate-saline medium has little immediate effect on the respiration as measured by the direct Warburg technic. However, in an extensive series of experiments, a slight increase of oxygen consumption in the presence of spermine was noted, especially during the third and fourth hour following the addition of the amine.

If glucose is present as a substrate for either brain brei or brain slices (cortex), the addition of spermine causes a marked inhibition of the oxygen consumption (for typical experiments see Table I, Exps. 11, 13, 81). This inhibition (calculated as % inhibition of the extra oxygen consumption due to glucose) shows a tendency to decrease with time, especially when brain slices are used. The averaged data from a series of 8 experiments on brain brei gave values of 75, 74, 73, and 65% inhibition of oxygen consumption by 0.0060 M spermine in each of the 4 thirty-minute periods following addition of the amine. With 0.0015 M spermine, the average inhibition for the same periods was 62, 57, 54, and 51% respectively. Spermine also inhibits the respiration of brain brei in the presence of blood serum as a substrate (Table I, Exp. 53). Similar inhibi-

* These investigations were aided in part by grants from the Rockefeller Foundation and from Armour and Company.

tion is observed with lactate and pyruvate as substrates (Table I, Exps. 27, 29, 37, 40). At the concentrations used the amine has relatively small effect on the extra oxygen consumption due to the addition of glutamic acid (Table I, Exps. 25, 26). With succinic acid no appreciable effect is demonstrable (Table I, Exp. 15).

The addition of small amounts of spermine to guinea-pig skeletal muscle *in vitro* likewise causes respiratory inhibition although this occurs in the later periods of the experiment. Table II shows the effect of varying concentrations of the amine on the respiration of minced muscle suspended in phosphate buffer containing citric acid and boiled muscle extract. The inhibition can be demonstrated in the absence of both the citrate and the muscle extract, but the total respiration of the tissue is then much smaller. Preliminary experiments indicate that a similar inhibition occurs with strained liver suspensions.

The effect of spermine on glucose, lactate, and pyruvate oxidations in brain superficially resembles the action of various narcotics¹ although spermine does not produce narcosis in the intact animal. Since the oxidative inhibition of the narcotics has been associated with an inhibition of the tissue dehydrogenases, we investigated the possible effect of spermine on these enzymes. Determination of the rate of decoloration of methylene blue (Thunberg technic) by buffered brain brei indicates that spermine causes a small but definite acceleration (ca 20%) in the rate of decoloration, both in the presence and absence of glucose.

The *in vitro* action of spermine on brain oxidations also resembles that of certain amines, notably tyramine, β -phenyl-ethylamine, β -phenyl- β -hydroxy-ethylamine, and mescaline.² Neither these substances nor the narcotics are regarded as normal tissue constituents. The facts here presented are of special interest, therefore, in that they demonstrate that spermine, a normal constituent of brain as well as other tissues, has *in vitro* properties similar to those of various pharmacological agents acting on the central nervous system.

Since the systems inhibited by spermine seem to be those which require the presence of co-enzymes, it is possible that spermine may act by interfering with co-enzyme action. This possibility is being investigated.

Associated with spermine in the brain, pancreas, and iris, is a chemically related base, spermidine (N-propylamino-diaminobu-

¹ Quastel, J. H., and Wheatly, A. H. M., *Proc. Roy. Soc.*, B, 1932, **112**, 60.

² Quastel, J. H., and Wheatly, A. H. M., *Biochem. J.*, 1933, **27**, 1609.

TABLE I.

Tissue	Substrate	Exp. No.	Molar Conc. Spermine	Time, min.	mm ³ O ₂ consumed per 100 mg wet wt.—				mm ³ O ₂ consumed per 100 mg wet wt.—				% Inhibition
					Brain	Brain + Spermine	Brain + Substrate	Brain + Spermine + Substrate	Brain	Brain + Spermine	Brain + Substrate	Brain + Spermine + Substrate	
Minced Guinea Pig Whole Brain	.0015 M Glucose	11	.0015	0-60	86.0	87.0	116.0	100.0	96.0	94.0	120.0	100.0	75
				60-90	34.0	36.0	54.1	44.9	33.9	35.0	47.1	38.9	70
				90-120	24.0	27.0	39.9	37.1	25.3	32.2	42.9	39.1	61
	.013 M Lactate	29	.0015	0-60	72.0	71.4	120.1	102.5	72.8	70.9	118.6	93.1	52
				60-90	24.9	26.8	47.8	40.3	24.1	28.5	48.8	37.7	63
				90-120	21.9	22.0	41.7	33.4	22.3	24.6	40.8	31.5	63
	.013 M Pyruvate	37	.0015	0-60	69.1	74.1	130.2	110.8	66.7	68.4	126.2	92.8	59
				60-90	24.0	25.6	50.3	38.2	26.0	24.6	50.8	31.8	71
				90-120	21.7	21.0	42.4	33.0	17.3	24.0	42.8	31.8	69
	.053 M Glutamate	25	.0015	0-60	76.4	73.7	102.1	98.9	68.4	68.5	102.0	96.3	17
				60-90	22.8	25.5	42.0	40.0	23.6	26.3	44.1	40.6	30
				90-120	19.3	21.1	34.3	36.1	24.5	28.1	40.0	37.4	40
Guinea Pig (Cortex) Brain slices	.021 M Succinate	15	.0030	0-60	66.5	65.5	92.9	91.7	1				
				60-90	27.0	26.1	38.1	38.5	—12				
				90-120	20.4	22.6	32.2	33.3	9				
	10% guinea pig blood serum	53	.0060	0-60	65.3	73.2	126.6	95.1	64				
				60-90	16.7	22.1	34.5	26.3	76				
				90-120	13.3	24.9	38.1	30.5	77				
	.0015 M Glucose	81*	.0015	0-60	6.38	7.04	16.02	11.59	53				
				60-90	1.33	1.84	6.49	5.86	41				
				90-120	1.15	1.60	4.81	4.69	16				

Contents of vessels: 0.7 cc M/5 phosphate buffer, pH 7.4 + 1.1 cc 0.9% NaCl + 100 mg wet tissue. Other constituents as indicated, all added as neutral salts. 0.1 cc 20% KOH and starch-free filter paper in central cups.

* 0.7 cc M/15 phosphate buffer used. O₂ consumption as mm³ O₂ per mg dry weight of tissue.

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tane). Our preliminary studies indicate that this substance possesses properties similar to those reported here for spermine.

TABLE II.

Time, min.	mm ³ O ₂ consumed*			
	Muscle	Muscle + .0037 M Spermine	Muscle + .0019 M Spermine	Muscle + .00027 M Spermine
0-60	540	517	528	497
60-120	378	210	338	358
120-180	165	33	84	139
180-240	32	21	23	27
Total O ₂ consumption	1115	781	973	1021
Inhibition		30%	13%	8%

* Each vessel contains 1.6 cc muscle suspension (cooled guinea pig leg muscle minced and suspended in 5 vl. M/10 phosphate buffer, pH 6.8) + 1.0 cc muscle extract (guinea pig leg muscle minced + 1 volume water, kept in boiling water bath 10 minutes and filtered) + .3 cc 0.2 M sodium citrate + 0.2 cc spermine hydrochloride in 0.9 % NaCl made up to final molarities.

10713 P

Studies on the Colloidal Gold Curve of Blood Serum in Liver Disease.

SEYMOUR J. GRAY. (Introduced by George F. Dick.)

From the Department of Medicine, University of Chicago.

The quantitative alterations of the plasma proteins in liver disease have been recognized for many years. The work of Gros¹, Kendall² and de Vries³ suggests, however, that a qualitative change in the plasma globulin, as indicated by an increase in the euglobulin fraction, may occur frequently in hepatic disease. These investigations indicate that an increase in euglobulin may distinguish the globulin of liver disease from that of other diseases. Since the plasma globulin⁴ and, particularly, the euglobulin⁵ have been shown to play an important rôle in colloidal gold precipitation, these studies of the colloidal gold reaction of blood serum in liver disease were undertaken.

¹ Gros, W., *Deutsch. Arch. f. klin. Med.*, 1935, **177**, 461.

² Kendall, F. E., *J. Clin. Investigation*, 1937, **16**, 921.

³ de Vries, A., *Act. med. Scandinav.*, 1938, **98**, 95.

⁴ Cruickshank, J., *Brit. J. Exp. Path.*, 1920, **1**, 71.

⁵ Mellanby, J., and Anwyl Davies, T., *Brit. J. Exp. Path.*, 1923, **4**, 132.

The method is essentially the same as that of the Lange test on spinal fluid except for serum dilution, salt concentration and acidity of the colloidal gold solution. One tenth of a cubic centimeter of the patient's blood serum is diluted to 1:350 with 0.9% sodium chloride, and serial dilutions are made as in the Lange reaction, using 0.3% sodium chloride in the ten tubes. Five cubic centimeters of colloidal gold, properly acidified, are added to each tube and the reactions are read in 12-24 hours. The colloidal gold solution is the same as that used routinely in the serology laboratory. The colloidal gold solution is acidified with 1/50 normal hydrochloric acid, and the degree of acidity is determined by testing the solutions with sera from normal patients and from patients with obvious liver disease. Standardization of acidity is done only once for each supply of colloidal gold prepared. The acid is added dropwise immediately before each test is performed. Usually 1.1 to 1.6 cc of 1/50 normal hydrochloric acid are added to every 50 cc of colloidal gold used. The pH is about 7.12 varying with the individual colloidal gold preparations. In reading the test the same numbers are used as in reading the spinal fluid Lange reaction. The greater the precipitation of colloidal gold the higher the number. Number 5 represents complete precipitation. The range of normal was found to vary from 0000000000 to 3332210000. The positive reaction was found to be a paretic curve consisting of complete precipitation⁵ or incomplete precipitation⁴ in one or more of the tubes on the left side of the curve as 5532110000 or 4432110000.

The colloidal gold reaction of blood serum was studied in 46 cases of liver cirrhosis, 14 cases of acute parenchymatous hepatic disease, 25 cases of neoplastic liver involvement and 11 cases of miscellaneous liver disease in which autopsy, biopsy or laparotomy confirmed the diagnosis, in 11 cases of the first group, 2 cases of the second, 13 cases of the third and 8 cases of the fourth.

Positive serum colloidal gold reactions, as indicated by paretic curves, were obtained in each of 46 cases of hepatic cirrhosis, in 13 of 14 cases of acute parenchymatous liver involvement, in 19 of 25 cases of hepatic neoplasm and in each of 11 cases of miscellaneous liver disease. The reaction was positive in 89 of 96 cases, or 92.7% in the total group of liver disease in which the diagnosis was confirmed by autopsy, biopsy or laparotomy in 34 cases. The serum colloidal gold reaction was negative in 20 normal adults and in 73 of 75 patients with various extrahepatic diseases. Autopsy, biopsy or laparotomy confirmed the presence of normal livers in 22 cases of this group.

The mechanism of the serum colloidal gold reaction in liver disease is not known. Positive reactions do not depend primarily upon a quantitative increase in globulin, lowering of albumin or inversion of the albumin-globulin ratio. Positive colloidal gold reactions were present in 29 of the 32 cases with normal plasma globulin determinations and in 21 of the 23 cases with normal albumin-globulin ratios. Conversely in the control series the colloidal gold reaction was negative in 13 of the 15 cases with elevated plasma globulin values and in each of the 15 cases with low or inverted albumin-globulin ratios. It is suggested that since the euglobulin is believed to play an important rôle in colloidal gold precipitation and is thought to be specifically increased in liver disease, it may be responsible for the positive reactions obtained.

10714 P

The Relationship of the P-P Factor to Gastrointestinal Motility.

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AND JEAN DUNBAR.

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Medical School, Chicago.*

In a previous preliminary report we have outlined our observations on the effect of whole vitamin B complex¹ and of some of its components on functional digestive disturbances. We have studied 65 cases in which the diagnosis of functional digestive tract disorder was made after every attempt to reveal any existing pathology by X-ray examination and appropriate laboratory tests. The principal complaints of these patients have been distress, flatulence (bloating, passage of flatus, borborygmi), and constipation which might alternate with loose stools. Less constant symptoms are anorexia, weakness and fatigue, and nervousness. A group of 45 have been treated over periods of 2 months to 2 years with whole vitamin B complex; 70% of these have shown a satisfactory response to this therapy. The presence of a vitamin B deficiency in this group of individuals was indicated by determinations of urinary thiamin output, which was below normal in 17 of 22 patients. (That is, below 10 I.U. per day.)

¹ Crandall, L. A., Jr., and Chesley, F. F., *Proc. Inst. of Med., Chicago*, 1939, **12**, 359.

In parallel determinations on a group of 16 normal individuals the average excretion was 20.5 I.U. per day.

Groups of patients were treated separately with thiamin, riboflavin, and nicotinic acid to determine whether any of these fractions of the vitamin B complex could be shown to be responsible for its effectiveness. Negative results were obtained with thiamin and riboflavin. However, in 40 cases treated with nicotinic acid in dosages ranging from 75 to 200 mg per day, the beneficial effects obtained were comparable to those resulting from treatment with whole complex (satisfactory in 60%). It is our impression that the therapeutic effect of nicotinic acid in functional bowel disturbance is not the complete equivalent of that given by the whole B complex, but further study may be necessary to establish this point.

A marked intestinal hypermotility was noted in several of our patients on roentgen ray examination. This observation, together with the digestive disturbances that are known to occur in human pellagra and canine black tongue, led us to examine the intestinal motility of dogs on a black tongue-producing diet. Six animals have been thus studied. The dogs were placed on a complete diet plus additional amounts of yeast extract for 2 weeks prior to starting the black-tongue diet. Roentgen ray observations were made by giving the animals 30 g of barium in 150 cc of water, noting the gastric emptying time fluoroscopically, and following the progress of the barium through the small and large intestines by films taken at the end of the second hour and every hour thereafter for 5 hours. The experimental diet consisted of corn meal 80, washed casein 10, peanut oil 5, cod liver oil 2, and $\text{Ca}_3(\text{PO}_4)_2$, CaCO_3 , and NaCl 1 each. Evident disturbances of motility occurred in all of the 6 animals studied. Hypermotility was the most common abnormality, and tended to occur within 3 to 5 weeks after the dogs had been started on the black-tongue diet. At about the same time changes in the small intestine pattern, consisting of areas of apparently atonic small bowel in which barium collected and which were separated by presumably spastic regions, appeared. These changes were comparable to the "puddling" which has been noted in human small bowel patterns as the result of deficiency diseases. Accumulations of small bowel gas, which is rarely noted in normal animals, were observed in 3 of the 6 dogs; this was accompanied by increased amounts of gas in the large intestine. In the 3 animals showing the greatest intestinal hypermotility, barium reached the rectum in 2 hours. In 2 instances this extremely rapid propulsion of the barium meal appeared just prior to the development of black-tongue; in the third animal it occurred 3 weeks after an attack of black-tongue

which had been treated by a single injection of nicotinic acid (50 mg intravenously) while the animal remained on the experimental diet.

The periods of hypermotility were interrupted in 3 of our 6 animals by other periods of hypomotility. Whether hyper- or hypomotility was present, however, the entire gastrointestinal tract was involved so that gastric emptying time, small bowel passage time, and propulsion in the large intestine were all affected. The large bowel appeared the least susceptible to the effects of the deficiency.

It is our impression that the P-P factor (nicotinic acid, nicotinamide, or any carboxypyridine derivative capable of replacing these in the diet) is essential to the maintenance of normal gastrointestinal motility, and that absence of this factor leads to motor dysfunction of the intestinal tract prior to the appearance of the usually accepted deficiency symptoms. We base this opinion on the response of many cases of functional digestive disturbance in the human, some of which have shown hypermotility, to nicotinic acid; and on the abnormal intestinal motility appearing in dogs on a black-tongue-producing diet long before mouth lesions were evident. Although we have not been able to demonstrate any effectiveness of thiamin or riboflavin in functional digestive disturbances, it is entirely possible that other factors in the vitamin B complex may be concerned with gastrointestinal function.

10715

Persistence of Medullary Tissue in Homotransplanted Adrenals.

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Observations by the senior author¹ indicated that the anterior chamber of the eye was a favorable site for the incorporation and persistence of adrenal cortices in the rat. Out of 243 grafts persisting subsequent to insertion of whole glands, medullary tissue was observed in only one instance. Since it was possible to increase the incidence of medullary persistence by removal of the surrounding cortex previous to insertion into the eyes, it was postulated that the survival of medullary tissue was conditioned by the rapidity of vascularization or by the accessibility of nutritional substances. In

¹ Turner, C. Donnell, *Anat. Rec.*, 1939, **73**, 145.

view of the current interest²⁻⁴ in the viability of endocrine glands following disruption of their normal innervation, these studies on the adrenal medulla were pursued further by transplantations into the kidney.

Whole adrenal glands from newborn rabbits were transplanted bilaterally into the eyes of 6 adult female hosts. The grafts were frequently observed macroscopically through the corneas and were removed for histological examination after persistence for 60 days. Two grafts from one host were found highly fibrotic and no traces of adrenal tissue were detectable. All of the remaining grafts contained cortices (Fig. 1) and the disposition of the cortical cells approximated that characteristic of normal intact glands. Seven of 10 non-fibrotic grafts possessed medullary tissue (Fig. 1).

We find that whole adrenal glands from newborn mice incorporate and persist well when inserted underneath the kidney capsule of the mother. To this date 47 out of approximately 55 such grafts have been recovered after persistence for periods varying from 2 days to 4 months. Thirty-two transplants of this type (68%) have been found to possess variable amounts of medullary tissue (Fig. 2). In some cases the extent and histological character of the medullary tissue in the grafts did not differ appreciably from that present in intact glands from animals of comparable ages. In the adrenals of the mouse the medulla approaches the exterior in the region of the hilus, and we believe that this is a factor which facilitates rapid vascularization and hence medullary incorporation in this species. In our best instances of medullary persistence the hilus of the gland has been pressed against the substance of the kidney. So far none of the transplants have contained medulla when the glands were inserted with the hilus away from the kidney tissue, *i. e.*, pressed against the kidney capsule.

Summary. Seven out of 12 whole adrenal glands from newborn rabbits contained medullary tissue after being homoplastically transplanted into the anterior chambers of adult female hosts. Under the conditions herewith described, 68% of adrenal homotransplants into the kidney of the mouse contained medullary tissue for from 2 days to 4 months after transplantation. The observations are interpreted as indicating that the survival of the medullae in adrenal transplants is conditioned, in part, by opportunities for rapid vascularization or

² Haterius, H. O., Schweizer, M., and Charipper, H. A., *Endocrinol.*, 1935, **19**, 673.

³ Haterius, H. O., *Cold Spring Harbor Symposia on Quantitative Biology*, 1937, **5**, 280.

⁴ Fisher, C., Ingram, W. R., and Ranson, S. W., *Monograph on Diabetes Insipidus*, 1938.

by the accessibility of nutritional body fluids. The functional capacity of the medullary tissue in our transplants has not been demonstrated.



FIG. 1.

Adrenal gland from a newborn rabbit 60 days after homotransplantation in the anterior chamber of the eye of an adult female host. The cortico-medullary boundary has been outlined with ink.

FIG. 2.

An adrenal 3 months after homotransplantation in the kidney of an adult mouse. Note the persistence of the medulla and the position of the hilus with reference to the kidney substance. The cortico-medullary boundary has been outlined with ink.

10716 P

Transmission of Reagin Through the Placenta.

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Placental permeability to foreign proteins was first demonstrated by Ascoli¹ who recovered, by precipitin reactions, from the rabbit fetal circulation egg white and horse serum that had been injected parenterally into the mother. Later, passage of anaphylactic antibodies through the guinea pig placenta was demonstrated by Rosenau and Anderson² and by Otto.³ Subsequently transmission of diphtheria and tetanus antitoxin from mother to child was shown to occur in humans.^{4, 5}

Insofar as we know, no one has demonstrated placental transmission of the reagin from hypersensitive mother to her offspring. Bell and Eriksson were unable to demonstrate specific reagins in newly born infants of allergic mothers.⁶ Since the multitude of factors concerned in this process necessarily makes control of the experiment difficult, it was decided to attempt the procedure in the Rhesus monkey.

A *Macacus rhesus* monkey whose sex cycle had been completely studied by the vaginal smear method was impregnated. The exact time of exposure being known, the expected date of delivery was calculated as 166 days later. Ten days prior to delivery the mother's skin did not react in sites locally sensitized with pea-wheat-rye reaginic serum from a hypersensitive patient. Seven days before delivery 20 cc of the pea-wheat-rye reaginic serum was injected subcutaneously and the injection was repeated daily. After having had 80 cc of the serum, positive skin tests to pea and wheat appeared and just before delivery when 140 cc had been administered large wheals could be produced with pea and wheat and a test to

* The expense of this investigation was borne in part by a grant from Parke, Davis and Company.

¹ Ascoli, A. Z., *Phys. Chem.*, 1902, **36**, 498.

² Rosenau, J. J., and Anderson, J. F., *Hyg. Lab. Bull.*, 1906, 29, U. S. P. H. Service.

³ Otto, R., *Münch. med. Woch.*, 1907, **54**, 1665.

⁴ Polano, O., *Z. f. Geburtsh. u. Gynäk.*, 1904, **53**, 456.

⁵ Ten Broeck, C., and Bauer, J. H., *PROC. SOC. EXP. BIOL. AND MED.*, 1923, **20**, 399.

⁶ Bell, S. D., and Eriksson, Z., *J. Immunol.*, 1931, **20**, 447.

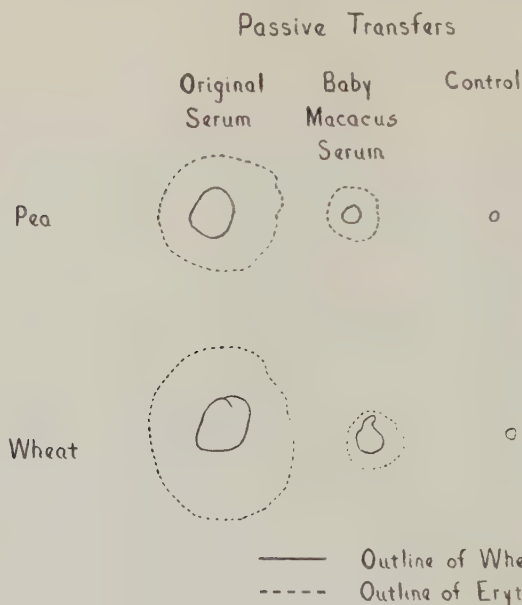


FIG. 1.

rye could also be demonstrated. After delivery the baby was bled and tested with the same antigens. Direct tests were all negative, Passive transfer of the baby's serum to human skin gave positive reactions to pea and wheat.

We have been able to transfer the reagin of hypersensitive man through the placenta of the *Macacus rhesus* monkey. Since the *Macacus rhesus* monkey accepts passive transfer like man,⁷ there is a possibility that this mechanism of transfer exists in man.

⁷ Strauss, H. W., *J. Immunol.*, 1937, **32**, 251.

10717 P

Proliferation of Myeloid and Lymphoid Cells Induced by Extracts of Urine from Leucemic Patients.*

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Leucemias occur spontaneously in many different species of animals. Mammalian leucemia has been transmittted from one animal to other animals by living cell transplants.^{1, 2, 3} Avian leucemia has been transmitted to other animals of the same species with cell-free, bacteria-free filtrates.^{4, 5} Successful transmission of human leucemia to humans or other animals has never been accomplished.

The experiments reported here were performed to test whether urine from human individuals with leucemia might contain some agent capable of stimulating the reticulo-endothelial system of other animals.

Urines were collected from patients with chronic myeloid leucemia, chronic lymphoid leucemia, subacute monocytic leucemia, Hodgkin's disease, multiple myeloma, carcinoma, and aplastic anemia. Urine was also collected from normal individuals. Extracts of these urines were prepared by adsorption on Kaolin and elution with alcohol after the method of Houssay and Biasotti.⁶ Their method was modified only by reducing the pH of the urine to 1.5-3.0 before adsorbing on Kaolin and with the adjustment of the pH of the final aqueous solution to neutral. This solution, after filtration through Berkefeld V or Seitz filters, was given to rats, mice, guinea pigs, and monkeys, but this report deals only with the results in guinea pigs.

Thirty-four guinea pigs received the extract from the urines of patients with chronic myeloid leucemia in doses of from 2-4 cc twice daily. The majority of the animals were ill or died within 6 weeks, although some showed effects of the injections as early as 2 weeks, and others not before 4 months. Usually at about 5 to 6

* This research has been aided, recently, by a grant from the Commonwealth Fund.

¹ Gie, Tio Tjwan, *Amsterdam Acad. Proefschr.*, 1927.

² Richter, M. N., and McDowell, E. C., *J. Exp. Med.*, 1930, **51**, 659.

³ Furth, J., Seibold, H. R., and Rathbone, R. R., *Am. J. Cancer*, 1933, **19**, 521.

⁴ Ellerman, V., and Bang, O., *Centr. f. Bakt.*, 1908, **46**, 4.

⁵ Furth, J., *J. Exp. Med.*, 1931, **53**, 243.

⁶ Houssay, B. A., and Biasotti, A., *Compt. rend. Soc. de biol.*, 1933, **133**, 469.

weeks the animals lost weight, had subcutaneous edema, and marked anemia. The total white blood counts generally were not elevated, but frequently during the latter part of the injection period myelocytes, myeloblasts, and nucleated red cells, as well as unidentified immature cells, appeared in the blood stream. Hyperplasia of the myeloid white cells was invariably present in the bone marrows. Sections of the spleens showed myeloid metaplasia, a destruction of the splenic architecture, and reduction in size and number of the normal splenic follicles. Sections of the livers showed myelopoiesis in the parenchyma and frequently extravascular and periportal infiltration with myeloid cells. The adrenal, lungs, lymph nodes, bladder, body wall, and occasionally the heart muscle showed the same reaction in lesser degree. Only one of the 34 animals showed no reticulo-endothelial change.

The extracts of the urines from all of the patients with other conditions, as well as those from the normal individuals, exhibited no myeloid change in any organ. In this group there were 17 animals which received extracts from normal urine, 4 which received extracts from the urine of patients with chronic lymphoid leucemia, 2 which received that from an individual with multiple myeloma, and one each from the urines of individuals with aplastic anemia, subacute monocytic leucemia, Hodgkin's disease, and carcinoma. The animals which received extracts of urine from patients with chronic lymphoid leucemia and those from multiple myeloma did show hyperplasia of the lymphoid elements. The animal which received the extract from the urine of an individual with subacute monocytic leucemia and that which received the extract from the urine of a patient with aplastic anemia showed hyperplasia which we do not classify. Two blank controls were negative.

Summary. Myeloid metaplasia in liver, spleen, adrenal, and other organs, as well as myeloid white cell hyperplasia of the bone marrow, was produced in 33 guinea pigs by injections with extracts of urines obtained from patients with chronic myeloid leucemia. One animal, similarly treated, showed no change. Twenty-eight guinea pigs, injected with extracts of the urine from normal individuals and individuals with diseases other than myeloid leucemia, showed no myeloid change. The animals which received the extracts of urine from patients with chronic lymphoid leucemia and multiple myeloma showed lymphoid hyperplasia. Two animals showed hyperplasia which has not been classified.

10718 P

Simultaneous Observations of Pancreatic and Biliary Papillae of Rabbit.

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Since the pancreatic and biliary papillæ are widely separated anatomically in the rabbit, while these structures are closely connected in dog and man for example, the functional effects of various drugs on the biliary and pancreatic papillæ¹ may be studied with ease and certainty when each papilla is inspected by an observer at the same time.

All rabbits were narcotized by 300 mg of sodium barbital per kilo, injected subcutaneously; each papilla was inspected by one observer; only one drug was injected into each animal repeatedly, after intervals of not less than 30 minutes.

Crude secretin pH 7-8, 0.5 cc per kilo I.V., increased the sequential contraction¹ of both papillæ, but those observed in the pancreatic papilla were more frequent and persisted longer.

Purified secretin SI (Ivy's Method), 0.1 to 2 mg per kilo I. V., often increased the activity of both papillæ but the effect was more marked on the pancreatic than upon the biliary papilla; at times, the effect was limited to the pancreatic papilla.

Cholecystokinin (Ivy's A pH 1802 method), 8 mg per kilo I.V. has a similar effect to secretin S I.

Histamin hydrochloride, 10 to 200 γ per kilo I.V., generally increased the activity of the bile papilla more than that of the pancreatic papilla; occasionally the opposite effect was noted.

Acetylcholin chloride, 5 to 100 γ per kilo I.V. generally produced powerful contractions of the pancreatic papilla with little or no effect on the bile papilla.

Physostigmin sulphate, 150 γ per kilo I. V. increased the frequency and strength of contractions in both papillæ. The pancreatic papilla often exhibited a tonic contraction of the neck lasting 5 to 8 minutes; this prolongation was less marked in the biliary papilla.

Arecolin hydrobromide, 5 to 20 γ per kilo I.V. was generally more effective on the pancreatic papilla than upon the bile papilla; this increased action was largely limited to an increase in the rate, strength and duration of the neck contraction.

Epinephrin HCl, 50 to 100 γ per kilo I.V. abolished the spontaneous or induced activity of both papillæ.

¹ Auer, J., and Seager, L. D., *Proc. Soc. Exp. Biol. and Med.*, 1938, **39**, 542.

Atropin sulphate, 50γ per kilo I.V. stopped the activity of both papillæ.

In general, therefore, with the dosages noted, crude secretin, purified secretin, cholecystokinin, acetylcholin, and arecolin produce a greater effect on the pancreatic papilla of the rabbit than upon the biliary papilla; histamin affects the bile papilla more than the pancreatic papilla; and physostigmin increases the activity of both. Epinephrin and atropin abolish the activity of both papillæ.

10719 P

Observations on the Bovine Blood Picture in Health and Under Parasitism.

ELAINE DELAUNE. (Introduced by E. C. Faust.)

From Louisiana State University.

Observations made on 6 normal Jersey and Holstein calves between the ages of 2 days and 6 months showed an average of 8.70 million red cells, 10,674 white cells per cubic millimeter of blood and a differential leucocytic count as follows: Lymphocytes, 64.4%; monocytes, 12.2%; neutrophiles, 19.6%; eosinophiles, 3.3%.

Six counts made on each of 5 adult animals between the ages of 3 years and 6½ years, which were on range and which were considered to be normal from the standpoint of the absence of disease, revealed an average of 6.39 million red cells and 10,225 white cells per cubic millimeter of blood, with a differential leucocytic count of: Lymphocytes, 58.1%; monocytes, 8.0%; neutrophiles, 25.9%; eosinophiles, 7.0%.

The feeding of a pure nodularworm culture produced in calf No. 95 a sharp drop of 2½ million red cells 5 days after the administration of the larvæ, with an increase of 5000 in the total number of white cells caused by an increase above the previous range of 17% in the number of neutrophiles. These general changes were of constant occurrence, though not so sharply shown in the 6 individuals which had a mixed infection of hookworms (*Bunostomum phlebotomum*) and nodularworms (*Oesophagostomum radiatum*) as in the 2 animals with the pure nodularworm infection. The calves used in all these experiments were kept under controlled conditions preceding and during the period of infection. For each experimentally parasitized calf, a control calf was kept under similar conditions.

The same picture found in experimentally-produced cases of parasitism was noted in a naturally infected Brahma heifer about 1½ years old. This animal was brought to the laboratory in a much weakened condition, greatly emaciated, and passing very small quantities of liquid fecal material with a very fetid odor. Fecal examinations during the entire time of observations revealed a very high number of parasite eggs. This animal showed the characteristic drop in the number of red cells to 5.25 million, with a phenomenal rise in the white cells to 31,000 correlated with an increase of 35% in the neutrophiles, which reached its maximum 24 hours before death.

10720 P

Cultivation of Certain Viruses Using Yolk of Chick Embryo as Route of Injection.

FRED D. STIMPert. (Introduced by John F. Kessel.)

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Cox¹ recently reported the use of the yolk sac of the developing chick embryo as a medium for growing rickettsiae of Rocky Mountain spotted fever and typhus fever. Success was also attained in the cultivation of Eastern and Western strains of equine encephalomyelitis by the same worker (personal communication May 2, 1939).

This preliminary report confirms the results of Cox with the Eastern and Western strains of equine encephalomyelitis virus and in addition summarizes the results obtained with the virus of St. Louis encephalitis and 3 strains of poliomyelitis virus recovered locally.

In essentials the method employed is the same as that described by Cox. Fertile eggs were incubated at 39°C until injected and then at 37.5°C for the cultivation studies. Injections of the inoculum in doses of 0.1 to 0.5 cc were made directly into the yolk through a small opening in the air sac end of the egg which would just allow the passage of a 21 gauge needle. The opening was then closed with melted paraffin. Three to 4 eggs were used for the same inoculum in each passage and infectivity tests of the embryonic tissues, ex-

* Aided by contributions to the Bacteriology Research Fund and a grant from the National Foundation for Infantile Paralysis.

¹ Cox, H. R., *Public Health Reports*, 1938, **53**, 2241.

cept when titrating for end point infectivity, were made from 10% ground suspensions. Intracerebral doses of 1.0 cc were given to Rhesus monkeys for testing infectivity in the experiments with poliomyelitis virus and 0.03 cc given to white mice for the other viruses. One monkey as compared with 2 to 4 mice was used for each suspension as required.

Three series of egg passages, represented by different doses of original infective mouse brain inoculum—0.1, 0.2, and 0.5 cc were maintained for each of the following viruses: Eastern equine encephalomyelitis, Western equine encephalomyelitis and St. Louis encephalitis. Six-day eggs were used and the embryonic tissues were harvested and pooled for infectivity tests and egg passage 3 days after injection. Embryo tissue of the second egg passage of Eastern equine encephalomyelitis virus was infectious in mice in dilution of 1-100,000. The 1-500,000 dilution was negative in mice but when transferred to the third egg passage the pooled embryonic tissues of this passage produced fatal infection in mice. Yolk sac tissue was infectious in mice in the dilution of 1-10,000 but when transferred to the third egg passage the infectivity was lost. The Western equine encephalomyelitis virus has not been titrated from egg passage as yet but the embryonic tissues were infectious in the 0.1 cc and 0.2 cc dose series through the fourth passage in the usual mouse dose. The St. Louis encephalitis virus has maintained constant infectivity in the 0.1 cc dose series through the ninth passage and the 0.2 cc dose series through the seventh passage. This virus has not been titrated to end-point infectivity but was found infectious in the eighth passage in dilution of 1-1000. In all cases the embryos were dead when harvested on the third day and it is suggested in future work that the tissues be taken earlier. It is of interest to note that in the 0.5 cc dose series of original inoculum the infectivity was lost in the second or third passage in each case. This may possibly have resulted from very early death of the embryo by use of the large dose.

The strains of poliomyelitis virus selected were the McS, which produces experimental poliomyelitis of moderate severity; the BK, which produces a more or less mild degree of severity, and the ST, which produces a severe form of the disease. Each of these strains has been carried in 12 or more monkey passages and reported in previous work.^{2, 3} Four series of fertile eggs incubated 7, 8, 9, and 10 days respectively were injected with 0.5 cc of a 10% suspension of McS monkey cord (fresh, not glycerolated). Additional eggs in

² Stimpert, F. D., and Kessel, J. F., *Am. J. Hyg.*, 1939, **29**, 57.

³ Kessel, J. F., Stimpert, F. D., and Fisk, R. T., *Am. J. Hyg.*, 1939, **29**, 45.

each series were also injected with 1% and 0.1% suspensions of the same virus material. One series of 12-day eggs was injected with 0.2 cc doses of a 10% suspension of glycerolated BK virus cord pool. Two series, 9- and 12-day eggs were injected with 0.3 cc of a 10% suspension of glycerolated St virus cord pool. Three or more eggs were injected in each series and after 3 to 4 days' incubation the embryonic nervous tissue and the yolk sacs were recovered separately from each egg, pooled, ground and injected into monkeys to determine infectivity. All results were negative in the 22 monkeys employed. Control animals given the virus suspensions used in the egg injections showed typical infection.

10721

A Spectrophotometric Method for the Study of Fat Transport and Phosphorylation*

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The study of fat transport has been facilitated by the use of labelled molecules whose fate in the animal body could be followed. The tracers thus applied to fat transport studies have been: iodized fatty acids,¹ elaidic acid,² deuterium,³ and the radioactive isotope of phosphorus.⁴ Miller and Burr⁵ have followed the transport and distribution of tung oil in rats by utilizing the characteristic absorption band of eleostearic acid which makes up more than 90% of tung oil. The characteristic absorption spectra of eleostearic acid, due to its 3 conjugated double bonds, makes possible the distinction of this acid from normal body fats by spectroscopic analysis. However, tung oil offers two disadvantages. First, it is poorly tolerated by animals. And second, one double bond may be selectively destroyed

* Aided by grants from the Rockefeller Foundation, and the Medical Research Fund of the Graduate School of the University of Minnesota. Assistance in the preparation of these materials was furnished by the personnel of Works Progress Administration Official Project No. 665-71-3-69, Sub-project No. 6714.

¹ Artom, C., *Arch. intern. physiol.*, 1933, **36**, 101.

² Sinclair, R. G., *J. Biol. Chem.*, 1935, **111**, 515.

³ Schoenheimer, R., and Rittenberg, D., *J. Biol. Chem.*, 1935, **111**, 163.

⁴ Hevesy, G., *Nature*, 1936, **136**, 754.

⁵ Miller, E. S., and Burr, G. O., *Proc. Soc. Exp. Biol. and Med.*, 1937, **36**, 726.

(by hydrogenation or some other method) giving rise to a new band at 2350 Å which may slightly affect the calculations. An activated corn oil has been found to overcome both difficulties.

Moore,⁶ and later Kass, Miller, and Burr,⁷ found that if fatty acids more unsaturated than oleic were subjected to prolonged saponification there was a marked increase in ultraviolet absorption due to a conjugation of the double bonds, and that the position of spectral absorption was dependent on the degree of unsaturation. Corn oil (Mazola), which contains a large amount of linoleic acid, but no acid more unsaturated than linoleic, gives rise to a single band at 2350 Å with an absorption coefficient, $E_{1\text{cm}}^{1\%}$ of about 500 when subjected to a modification of Moore's saponification treatment.⁸ Normal body fat has an $E_{1\text{cm}}^{1\%}$ at 2350 Å from about 5 to 15, depending on the tissue or organ from which it is extracted. After feeding this conjugated fat, any absorption at 2350 Å over the basal level for the particular tissue and fat fraction being studied will be due to tagged fat incorporated in the body fat. By accurate spectroscopic measurement at this wave length the exact amount of this fat present may be determined. Because of the great tendency for unsaturated fatty acids with conjugated double bonds to oxidize, certain modifications of the conventional methods of extraction and spectrophotometric analysis of tissue fats have been made.

Method. The tagged fat is fed by stomach tube in the form of the methyl or glycerol esters of the conjugated fatty acids of corn oil. After a predetermined interval of time the tissues to be examined are removed from the animal and immediately placed in a boiling water bath for 5 minutes. It has been found⁹ that enzymatic transfer of phospholipid fatty acids proceeds rapidly in certain excised tissues, and heating apparently stops this action. The tissue is then minced and frozen with solid carbon dioxide. The frozen tissues are then dried *in vacuo* at a pressure less than 1×10^{-4} mm Hg, ground fine, weighed and extracted with Bloor's mixture (3 parts alcohol and 1 part ether). This extraction and all subsequent evaporations must be carried out under nitrogen. A convenient way to accomplish this is to extract under a reflux condenser in a flask with a side arm. A constant stream of nitrogen is allowed to run in the side arm while the extraction is proceeding. After ex-

⁶ Moore, T., *Biochem. J.*, 1937, **31**, 138.

⁷ Kass, J. P., Miller, E. S., and Burr, G. O., *J. Biol. Chem.*, 1938, **123**, LXVI.

⁸ Kass, J. P., Miller, E. S., and Burr, G. O., unpublished data.

⁹ Barnes, R. H., Miller, E. S., and Burr, G. O., *PROC. SOC. EXP. BIOL. AND MED.*, in press.

tracting 1.5 to 2.0 hours the alcohol-ether mixture is evaporated under partial vacuum, a slow stream of nitrogen still running in the flask, and the residue extracted by washing several times with low boiling petroleum ether. The petroleum ether is filtered into a volumetric flask and evaporated *in vacuo*. The extract is then taken up in aldehyde-free ethyl ether and made to volume. An aliquot portion of the ether solution is taken for the determination of tagged fat in the "total fat extract." The remainder is evaporated almost to dryness and the phospholipids precipitated with acetone. For convenience and protection against exposure to atmospheric oxygen for longer than necessary, the precipitation is best carried out in a centrifuge tube and centrifuging rather than filtration used in separating the phospholipids. The acetone-soluble layer is poured off, washed, then evaporated and made up to volume in aldehyde-free ether. If the tissues are placed in a boiling water bath immediately after excision, and caution employed in preventing oxidation, the phospholipids will readily dissolve in ether and may then be made up to volume.

The aliquot portions of the acetone-soluble and insoluble fractions thus prepared are taken for spectrophotometric analysis. The remainder of the ether solutions is evaporated *in vacuo* and weighed. Spectral absorption measurements cannot be made on the weighed material because of the difficulty in evaporating to absolute dryness

TABLE I.

Fraction description	Results in percentages			
	Absorption time			
	5 min.		15 min.	
	Control	Iodoacetic acid	Control	Iodoacetic acid
1. Acetone-soluble extract in dry mucosa	17.2	13.9	18.7	24.1
2. Acetone-insoluble extract in dry mucosa	11.0	10.5	11.0	11.7
3. Total fat in dry mucosa (1 + 2)	28.2	24.4	29.7	35.8
4. Tagged fat in acetone-soluble extract	33.7	33.9	37.4	45.0
5. Acetone-insoluble fatty acids as tagged fat	0.72	0.90	1.4	1.3
6. Total fatty acids as tagged fat	26.7	25.4	30.7	34.6
7. Acetone-soluble tagged fat in dry mucosa	5.8	4.7	7.0	10.8
8. Acetone-insoluble tagged fat in dry mucosa	0.08	0.09	0.16	0.16
9. Total tagged fat in dry mucosa	7.5	6.2	9.1	12.4
10. Recovery $(7 + 8) \times 100 \div 9$	78.5	77.4	79.2	89.0

without some oxidation. The weights obtained after removal of the aliquot portions are used in the calculation of absorption coefficients and percentages of the extracts in the dried tissue by applying the proper dilution factor. Blank determinations must be made on the tissues of animals not receiving tagged fat, and these values subtracted from the experimental values.

Table I gives the results of a typical experiment. In this case the incorporation of tagged fat in the acetone-soluble and acetone-insoluble fat fractions of the mucosal tissue from the small intestine of rats was studied. An emulsion of the methyl esters of conjugated corn oil, bile salts, lipase (Pancreatin), and water was injected directly into the duodenum of fasted, male, albino rats. Some of them had iodoacetic acid included in the emulsion in a concentration of 1:10,000. Five and 15 minutes after the introduction of the fat emulsion the animals were etherized and their intestines washed out by the method of Barnes, *et al.*¹⁰ The washed intestines were carefully removed from the animals, placed on a glass plate, and the outside gently scraped with the edge of a microscope slide. In this way the intestinal mucosa was expelled from the intestine without contamination with intestinal muscle or mesenteric fat. The mucosa was immediately placed in a boiling water bath and the extraction and analysis carried out as has been described.

Calculations: The $E_{1\text{cm}}^{1\%}$ at 2350 Å of the tagged fat fed was 493 in this experiment. The blank $E_{1\text{cm}}^{1\%}$ for intestinal mucosa was: Total fat extract, 7; acetone-soluble, 6; and acetone-insoluble, 8. Then $[E_{1\text{cm}}^{1\%}(\text{acetone-soluble}) - 6] \times 100 \div 493 = \% \text{ tagged fat in acetone-soluble fraction (Row 4)}$. If both fatty acids of lecithin were tagged fat with an $E_{1\text{cm}}^{1\%}$ 493, then lecithin would have an $E_{1\text{cm}}^{1\%}$ 356. The percent of acetone-insoluble fatty acids, as tagged fat calculated on this basis, is $[E_{1\text{cm}}^{1\%}(\text{acetone-insoluble}) - 8] \times 100 \div 356$ (Row 5). In calculating the percent tagged fat in the total fat extract the denominator factor must be calculated for each extract and depends on the proportion of the total fat which is acetone-soluble and acetone-insoluble (Row 6). The percent tagged fat of the various fat fractions in the dry mucosa (Rows 7, 8, and 9) are calculated by multiplying the percent tagged fat in the different fat fractions by the percent of the respective fractions in the dry mucosa and dividing by 100. The percent acetone-soluble tagged fat (Row 7) plus the percent acetone-insoluble tagged fat (Row 8) should equal the percent total tagged fat in the dry mucosa (Row 9). As can be seen

¹⁰ Barnes, R. H., Wick, A. N., Miller, E. S., and MacKay, E. M., *Proc. Soc. Exp. Biol. and Med.*, 1939, **40**, 651.

in Row 10 these are approximately 20% low. This discrepancy is probably due in some part to errors in transfer, weighing, and spectrophotometric determinations, but can undoubtedly be accounted for in large measure by unavoidable oxidation. The magnitude and direction of the error has been found fairly constant, so the interpretation of data is not seriously affected.

The conjugated acids of corn oil used in this experiment had an $E_{1\text{cm}}^{1\%}$ of 493 at 2350 Å. Pure 10-12 linoleic acid has an $E_{1\text{cm}}^{1\%}$ 1200. Therefore approximately 54% of the fatty acids fed were no spectroscopically active. The calculations given above are based on the supposition that the spectroscopically active and inactive acids reacted alike in becoming incorporated with already existing body fat. This is purely an assumption and in order to express the data on the basis of spectroscopically active acids, 46% of the tagged fat values should be used.

Changes undoubtedly take place in the conjugated linoleic acid after remaining in the body for long periods of time. These changes might cause a loss of the configuration necessary for spectroscopic measurement, but still leave the intact carbon chain present in the body fat. For this reason all experiments using this tagged fat have been arbitrarily restricted to data collected not more than 6 hours after a single feeding of the fat.

Summary. By conjugating the double bonds of the linoleic acid of corn oil, a fat may be prepared which can be spectroscopically distinguished and quantitatively measured in body fat. A method for the extraction and determination of this tagged fat in animal tissues has been described.

Venous Circulatory Changes in the Abdomen and Lower Extremities Attending Intestinal Distention.

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Scott and Wangenstein^{1, 2}, observed that loss of blood into the infarcted gut in strangulating obstructions constituted an important factor in causing shock. It appeared equally significant to determine whether intestinal distention *per se* affects the circulation. To this end, the effect of intestinal distention upon the venous pressure in the lower extremities, the circulation time, the inferior vena cava, and the portal venous pressures was determined.

Method. (a) The first group of experiments was performed on healthy adult dogs under intravenous pentobarbital sodium (35 mg/kg) anesthesia, maintained by intermittent intramuscular administration. A small water-filled rubber balloon with a negligible pressure of its own was placed in the peritoneal space and connected to an external water manometer for recording of intraperitoneal pressure. The abdomen was then sutured closed. The sural vein to carotid circulation time was determined at intervals by the sodium cyanid method (Loevenhart, et al³). Air was injected per rectum in 5 and through the esophagus in 2 animals through a cervical esophagostomy. In this series no record was kept of the amount of air injected or of the intraluminal pressure.

(b) In a second series of animals, lateral venous pressures in the femoral or sural vein were determined by dividing the vessel and connecting the 2 segments to a small paraffined T-tube (Burton-Opitz⁴) which communicated with a saline manometer. The intraluminal pressure was measured by a manometer placed in the air injection system. Carotid blood pressure was obtained in the usual manner. Circulation times and intraperitoneal pressures were determined as in (a).

(c) In a third series of dogs, lateral pressure in the inferior

¹ Scott, H. G., and Wangenstein, O. H., *PROC. SOC. EXP. BIOL. AND MED.*, 1932, **29**, 744.

² Scott, H. G., *Arch. Surg.*, 1938, **36**, 816.

³ Loevenhart, A. S., Lorenz, W. F., Martin, H. G., and Malone, J. Y., *Arch. Int. Med.*, 1918, **21**, 109.

⁴ Burton-Opitz, R., *Am. J. Physiol.*, 1903, **9**, 198.

vena cava was determined by cannulation of the renal vein after nephrectomy, establishing connection with a saline manometer. This method was supplanted in later experiments by insertion of a No. 10 F or No. 12 F urethral catheter through the femoral vein up into the inferior vena cava, the catheter then being connected to the manometer. Carotid or femoral arterial blood pressures were obtained. Lateral portal venous pressure was observed by either (a) cannulating the divided splenic vein (Bayliss and Starling⁵), (b) cannulating the divided inferior mesenteric vein, or (c) insertion of a paraffined T-tube between the segments of the divided portal vein itself, the cannula in any case being connected to a saline manometer. The intraluminal, intraperitoneal, and saphenous (or femoral vein) pressures were recorded. In some experiments the intraluminal pressure was steadily increased; in others, the pressures were maintained at a predetermined level by a constant pressure (Perusse⁶) bottle. The duration of the experiments varied from 2 to 8 hours.

(d) To determine the effect of increased intestinal pressure on the mesenteric venous pressure itself, a small dog was anesthetized with intravenous pentobarbital sodium. The upper jejunum was divided and the distal stoma cannulated and connected to a water manometer and air injection system; the terminal ileum was ligated. Venous pressures were determined in different veins of the mesentery with each increase of intraluminal pressure.

(e) The ankle venous pressure using the method of Griffith, Chamberlain, and Kitchell⁷ and the circulation time from an ankle vein to the carotid were determined in 5 cases of clinical intestinal obstruction on admission to the hospital.

(f) The effect of opening the abdomen on the intraluminal pressure of distended bowel was studied in rabbits anesthetized with intravenous pentobarbital sodium. The terminal ileum was ligated, the duodenum cannulated and connected to the air injection system with a water manometer, and the abdomen sutured closed. The gut was then distended and the incision opened gradually, noting the pressures. The effects on the pressure of injecting or aspirating small quantities of air were recorded.

Results. (a) In the first series of dogs (Table I) distention was continued until respiration was almost entirely prevented and

⁵ Bayliss, W. M., and Starling, E. H., *J. Physiol.*, 1894, **16**, 159.

⁶ Perusse, G. L., *Surg., Gynec., and Obst.*, 1932, **54**, 770.

⁷ Griffith, G. C., Chamberlain, C. T., and Kitchell, J. R., *Am. J. Med. Sci.*, 1934, **187**, 371.

TABLE I.
Effects of Experimental Intestinal Distention on Sural Vein to Carotid Body
Circulation Time (Sodium Cyanid) and on Intraperitoneal Pressure.

Dog	Circulation time (seconds)			Intraperitoneal pressure increase (cm water)	Route of air injection
	Initial	Final	Increase		
1	14	33	19	8 ↔ 13	Rectum
2	42	48	6	6 ↔ 19	"
3	11	15	4	11 ↔ 14	"
4	11	19	8	2	"
5	17	135	118	6 ↔ 16	"
6	10	23	13	1 ↔ 2	Esophagus
7	13	87	74	14	"

cyanosis was present. An increase of circulation time from the lower extremity and an increase of intraperitoneal pressure was a constant accompaniment of the distention.

(b) In the second series of dogs (Table II) death occurred in each case from shock, being attended by dyspnea and cyanosis. In dog No. 4, shock appeared 6 hours after the distention was started. Elevation of the caudal extremity in steep Trendelenburg (45° downward tilt of the head) and transfusion with 500 cc of freshly drawn citrated blood revived the animal. It was sacrificed, however, and the bowel found to be badly discolored and engorged with blood, frank blood being found free in the peritoneal space. The venous pressure in the lower extremities steadily increased with increased intraluminal tension, a corresponding lengthening of circulation time being observed. In each animal a temporary rise of arterial blood pressure occurred with beginning distention.

(c) In the third series of dogs (Table III) the same rise of venous pressure in the lower extremities was found to accompany the increased intraperitoneal pressure attending the increased intraluminal pressure. Although no significant variation in inferior vena cava pressure was observed, the portal venous pressure underwent an early rise followed by a fall to a constant level. A temporary rise in arterial blood pressure corresponding to the temporary elevation in portal pressure was also observed, a chart of a sample experiment (dog No. 4, Table III) being shown in Figure 1.

(d) From Table IV it may be seen that a drop of mesenteric venous pressure accompanies increases of intraluminal pressure. When, however, the intraluminal pressure is increased from 50 to 100 cm water there is no change in the mesenteric venous pressure.

(e) In cases Nos. 2 and 3 of the clinical intestinal obstructions (Table V) the venous flow from the lower extremity was so retarded that the typical respiratory response to sodium cyanid could

TABLE II.
Effects of Experimental Intestinal Distention on Sural Vein to Carotid Body Circulation Time (Sodium Cyanid), Intraperitoneal Pressure, Carotid Blood Pressure and Venous Pressure in the Lower Extremities.

Dog	Duration of experiment	Intraluminal pressure increase (cm water)	Intraperitoneal pressure increase (cm water)	Initial consecutive arterial blood pressure (mm Hg) *	Initial and final venous pressure (cm water)	Circulation times consecutive determinations (seconds)
1	3 hr	30	36	(a) 105 (b) 125	2 47	
2	39 min	106	19 ↔ 24	(a) 135 (b) 150	3 17	(a) 15 (b) 24 (c) No typical response
3	35 min	130	16	(a) 120 (b) 170	3 11 (4.5 after deflation of bowel)	(a) 14 (b) 39 (c) 43
4	7 hr	180	35	(a) 140 (b) 180	4 58+	(a) 27 (b) 37 (c) 2 min after placing in steep Trendelenburg

* Consecutive 10- to 20-min determinations.

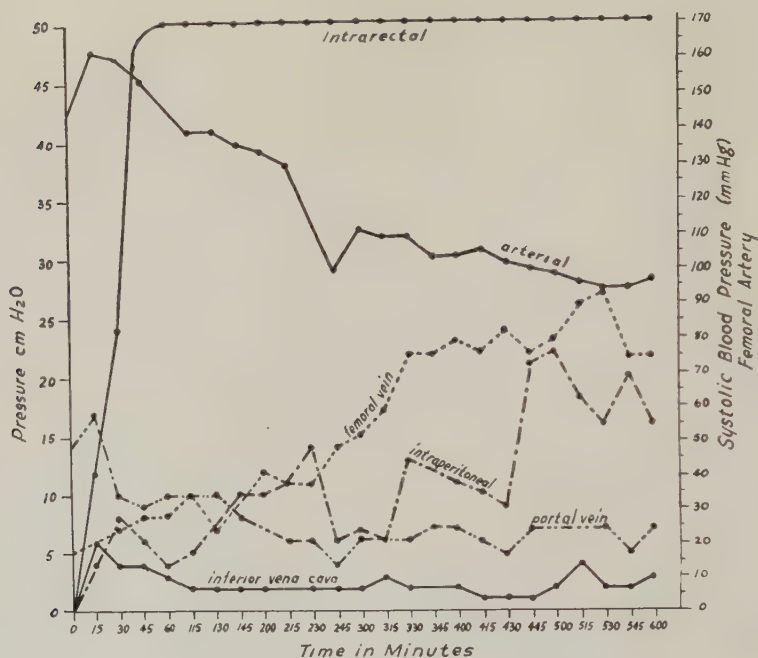


FIG. 1.

Intraintestinal Air Insufflation. Relation between arterial blood pressure, femoral venous pressure, inferior vena cava pressure, intraperitoneal pressure, and portal venous pressure. Intrajejunal pressure 50 cm water. (Table III, dog 4.)

only be elicited by placing the patient in the Trendelenburg position after injection into the ankle vein. The normal venous pressure and circulation time in case No. 4 is explained by the relatively small amount of distention found at operation.

(f) In a distended rabbit, when the intraluminal pressure was elevated to 104 cm water, opening the incision caused the pressure to fall to 84 cm water with rupture of the gut. In another animal the pressure was raised to 83 cm water, the tension dropping to 66 cm water when the abdomen was opened. In a third rabbit, aspiration and re-injection of 5 cc quantities of air into the distended bowel gave corresponding decreases and increases of intraluminal pressure of about 5 cc water. When the incision was opened 4 cm the intraluminal pressure dropped, additional pressure drops being noted when the incision was opened more. Exteriorization of bowel segments caused the intraluminal pressure to fall, exteriorization of

TABLE III.

Relation between Intraluminal, Intraperitoneal, Femoral Vein (or saphenous), Inferior Vena Cava, Carotid Artery (or femoral artery), and Portal Vein Pressures in Distended Dogs (bowel inflated with air).

Dog	Duration of experiment	Amount of air injected, cc	Arterial blood pressure, mm Hg*	Intra-luminal in gut, cm water	Intra-peritoneal, cm water	Femoral or saphenous vein,† cm water	Inferior vena cava, cm water	Portal vein pressure, cm water*
1	1 hr 55 min	2600	(a) 120 (b) Fall	0 100	0 10	3 43	8 (Through divided renal vein)	—
2	2 hr 25 min	3200	(a) 100 (b) Fall	0 202	0 18	10 31+	4 2 0 (Through divided renal vein)	—
3	6 hr	—	(a) 135 (b) 155 (c) Fall	100	0 10	7 21+	3 8 (Through catheter in femoral vein)	—
4	6 hr	—	(a) 144 (b) 162 (c) Fall	50	0	5 22	2 (Through catheter in femoral vein)	(a) 14 (b) 17 (c) 7 (Through divided inferior mesenteric vein)
5	8 hr 30 min	2250	(a) 110 (b) 115 (c) Fall	0 30	0 9	10 15	7 (Through catheter in femoral vein)	(a) 18 (b) 22 (c) 4 (Through divided splenic vein)
6	8 hr 15 min	—	(a) 110 (b) Fall	50	3 4	5 11	6 (Through catheter in femoral vein)	(a) 17 (b) 26 (c) 8 (T-tube in portal vein)

* Consecutive 10- to 20-min determinations.

† Initial and final readings.

TABLE IV.
Relation of Intraluminal Pressure in Bowel of Dogs to Mesenteric Venous Pressure
Attending Inflation of Gut with Air.

Intraluminal pressure (cm water)	Mesenteric venous pressure (cm water)
0	13
10	12
20	12
30	12
40	10
50	6
60	5.5
70	5
80	5
90	6
100	6

longer bowel segments giving additional pressure drops. This was confirmed in a fourth animal.

In the main, the experimental intraluminal pressures employed in this study exceeded considerably those occurring spontaneously in the course of the distention accompanying acute clinical intestinal obstructions. Observations made previously during the course of intestinal obstruction occurring in man, as well as in obstructions established experimentally in dogs, suggest that the ordinary range of sustained intraluminal pressures for the small bowel varies from 4 to 18 cm of water, 10 to 14 cm being usual. In acute obstructions of the colon in man, however, higher intraluminal pressures are the rule and pressures in excess of 20 are observed not uncommonly; on one occasion a pressure as high as 52 cm occurred (Sperling, Paine and Wangenstein⁸). The temporary increase of portal venous pressure and arterial blood pressure early in distention may be due to the sudden increased volume of blood forced from the intestinal vessels into the portal system, though a reflex vasomotor mechanism can not be excluded. The shock that occurs late in clinical intestinal obstructions and in the experimental distentions is due partly to the stasis in the vessels between the mesenteric arteries and the portal vein and partly to a mechanical impediment to the return of blood from the lower extremities. The increased pressure in the veins of the lower extremity and the lengthened circulation time in the experimental distentions are in accord with such a thesis. The circulatory stasis in the lower extremities and mesenteric area attending intestinal distention may interfere with the volume of circulating blood sufficiently to provoke shock unless relieved early by con-

⁸ Sperling, L., Paine, J. R., and Wangenstein, O. H., *PROC. SOC. EXP. BIOL. AND MED.*, 1935, **32**, 1504.

TABLE V.
Venous Pressure (Ankle Vein) and Circulation Time (Ankle Vein to Carotid Sinus by Sodium Cyanid Method) in Patients with Clinical Intestinal Distention.

Case		Venous pressure ankle (cm water)	Circulation time (seconds)	
			Cubital to carotid	Ankle vein to carotid
1	(Carcinoma of pelvic colon with acute obstruction; pressure at operation 20 cm water; 1800 cc gas aspirated)	12	20	52
2	(Carcinoma of pelvic colon; pressure at operation 15 cm water; 1500 cc gas aspirated)	15.5	19	No response in horizontal. 22" in 30° Trendelenburg*
3	(Carcinoma of stomach with distention owing to metastases)	17.5	18	No response in horizontal. 15" in 30° Trendelenburg*
4	(Mass in terminal ileum treated conservatively by suction; 5 days later ileostomy; intraluminal pressure 14 cm water; 600 cc gas and fluid aspirated at operation)	3	22	35
5	(Abdominal injury with marked intestinal distention treated conservatively by suction applied to indwelling duodenal tube)	29	12	49

* Downward inclination.

servative decompression (Wangensteen⁹). The Trendelenburg posture would appear to be an effective agency in interrupting the impediment to venous return from the lower extremities occasioned by abdominal distention.

Summary. 1. Experimental intestinal distention of anesthetized dogs produces an elevation of intraperitoneal pressure which, however, does not increase at the same rate as the intraluminal pressure. 2. Venous pressure in the lower extremities of experimentally distended, anesthetized dogs increases with increasing intraperitoneal pressure. At the same time, the rate of blood flow from the lower extremities to the carotid is slower. 3. Shock, eventually produced by prolonged intestinal distention, is relieved by deflation, blood transfusion and the steep Trendelenburg position. 4. Intestinal distention is attended by diminished respiratory movements, stasis of abdominal and peripheral venous blood, and extravascular loss of whole blood and plasma. 5. Pressure in the inferior vena cava is not observed to be altered in experimental intestinal distentions in which the intrainestinal pressures reached 50 cm of water—a pressure in excess of the upper limit of sustained intraluminal pressure observed clinically. 6. Following a temporary initial rise in portal venous pressure, a gradual fall to a constant low level is noted with continued experimental intestinal distention. 7. Early in intestinal distention, a temporary rise in arterial blood pressure is present. 8. With the abdomen open, increase of intraluminal pressure in the anesthetized dog results in a fall of mesenteric venous pressure to a constant level. 9. In clinical intestinal obstructions, there is an increase of venous pressure in and a delay in return of blood from the lower extremities. 10. Addition or aspiration of small quantities of air from the distended rabbit intestine causes corresponding increases or decreases of intraluminal pressure. Opening the abdomen or exteriorization of segments of bowel in such an animal causes fall in intraluminal pressure.

⁹ Wangenstein, O. H., *The Therapeutic Problem in Bowel Obstructions*, Springfield, Ill., 1937, Charles C. Thomas.

10723 P

Phenylalanine Content of Hen's Egg Albumin.

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The convincing evidence offered by Womack and Rose¹ that phenylalanine is essential in nutrition makes it desirable to know the phenylalanine content of the common food proteins. Moreover, a knowledge of the amino acid composition of purified proteins (or even of one purified protein) would be of immense value in formulating an adequate theory of protein structure. The last reported analysis of egg albumin for phenylalanine that the present authors have found was that of Osborne, Jones, and Leavenworth.² These authors, using the Fischer esterification method,³ obtained 5.07 g of phenylalanine from 100 g of egg albumin. Previous workers had reported 2.5% (Fischer⁴), 4.4% (Abderhalden and Pregl⁵), and 5.2% (Hongounenq and Morel⁶).

Egg albumin was isolated by the method of Cole.⁷ After 2 recrystallizations it was dialyzed in the apparatus described elsewhere.⁸ The protein was air-dried and kept in a desiccator over phosphorus pentoxide. Its nitrogen content was 15.13% (see Calvery⁹ and Arnow¹⁰); a solution of it in distilled water had a pH of 4.75; and 98% of it in solution could be coagulated by heat.

Phenylalanine was determined by a modification of the Kapeller-Adler¹¹ procedure. The results are summarized in Table I.

If egg albumin is assumed to have a molecular weight of 40,500 (Svedberg¹²), the data obtained suggest that one molecule of egg

¹ Womack, M., and Rose, W. C., *J. Biol. Chem.*, 1934, **107**, 449.

² Osborne, T. B., Jones, P. B., and Leavenworth, C. S., *Am. J. Physiol.*, 1909, **24**, 252.

³ Fischer, E., *Z. physiol. Chem.*, 1901, **33**, 151.

⁴ Fischer, E., *Z. physiol. Chem.*, 1901, **33**, 412.

⁵ Abderhalden, E., and Pregl, F., *Z. physiol. Chem.*, 1905, **46**, 24.

⁶ Quoted by Osborne, Jones and Leavenworth.

⁷ Cole, A. G., *Proc. Soc. Exp. Biol. and Med.*, 1933, **30**, 1162.

⁸ Bernhart, F. W., Arnow, L. E., and Bratton, A. C., *Ind. Eng. Chem. Anal. Ed.*, 1937, **9**, 387.

⁹ Calvery, H. O., *J. Biol. Chem.*, 1932, **94**, 613.

¹⁰ Arnow, L. E., *J. Biol. Chem.*, 1935, **110**, 43.

¹¹ Kapeller-Adler, R., *Biochem. Z.*, 1932, **252**, 185.

¹² Svedberg, T., *Ind. Eng. Chem. Anal. Ed.*, 1938, **10**, 113.

500 BLOOD PLASMA AND INTESTINAL FLUID DURING ABSORPTION

TABLE I.
Experimental Values for the Phenylalanine Content of Egg Albumin.

Quantity of egg albumin analyzed, mg	G of phenylalanine from 100 g of egg albumin, g	G of phenylalanine residue per 100 g of unhydrolyzed egg albumin, g
87.3	5.20	4.63
87.3	5.33	4.75
87.3	5.33	4.75
84.3	5.29	4.71
72.5	5.53	4.93
72.0	5.35	4.77
64.6	5.31	4.73
54.0	5.33	4.75
52.7	5.16	4.60
48.4	5.31	4.73
36.0	5.33	4.75
Avg	5.32	4.73

albumin contains 13 phenylalanine residues. On the other hand, adoption of the theory of Bergmann¹³ would make it appear probable that one molecule of protein contains 12 phenylalanine residues; and the calculated molecular weight of egg albumin would be 37,300.

10724 P

Osmotic Relationships Between Blood Plasma and Intestinal Fluid During Absorption.

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It has been noted by Katzenellenbogen,¹ Goldschmidt, and Dayton² and others^{3, 4, 5} that univalent ion salts may be absorbed from the intestine against concentration gradients. This paper is concerned with a study of the changes in vapor pressure or osmotic activity accompanying such absorption from the intestine of the dog.

Methods. Vapor pressure determinations were made with the

¹³ Bergmann, M., *Chem. Rev.*, 1938, **22**, 423.

¹ Katzenellenbogen, M., *Pflüger's Arch.*, 1906, **114**, 522.

² Goldschmidt, S., and Dayton, A. B., *Am. J. Physiol.*, 1919, **48**, 459.

³ Burns, H. S., and Visscher, M. B., *Ibid.*, 1934, **110**, 490.

⁴ Ingraham, R. C., and Visscher, M. B., *Ibid.*, 1936, **114**, 676.

⁵ Ingraham, R. C., and Visscher, M. B., *Ibid.*, 1936, **114**, 681.

thermo-electric method as modified by Baldes.⁶ This method involves a comparison of the rates of evaporation from or condensation on drops of the sample and of a reference solution when placed on opposite junctions of a thermocouple enclosed in a moist chamber. The deflection of the galvanometer to which the thermocouple is connected is a measure of the difference in temperature of the 2 drops, which in turn is a measure of the difference in vapor pressure of the 2 solutions. The results are expressed in terms of the concentration of the reference solution and since the vapor pressure of a solution varies inversely as the concentration, the term "osmotic activity" will be used in order to avoid confusion. Thus a solution with an osmotic activity of 150 mM NaCl is one in which the vapor pressure is equivalent to that of a solution containing 150 mM NaCl per kg

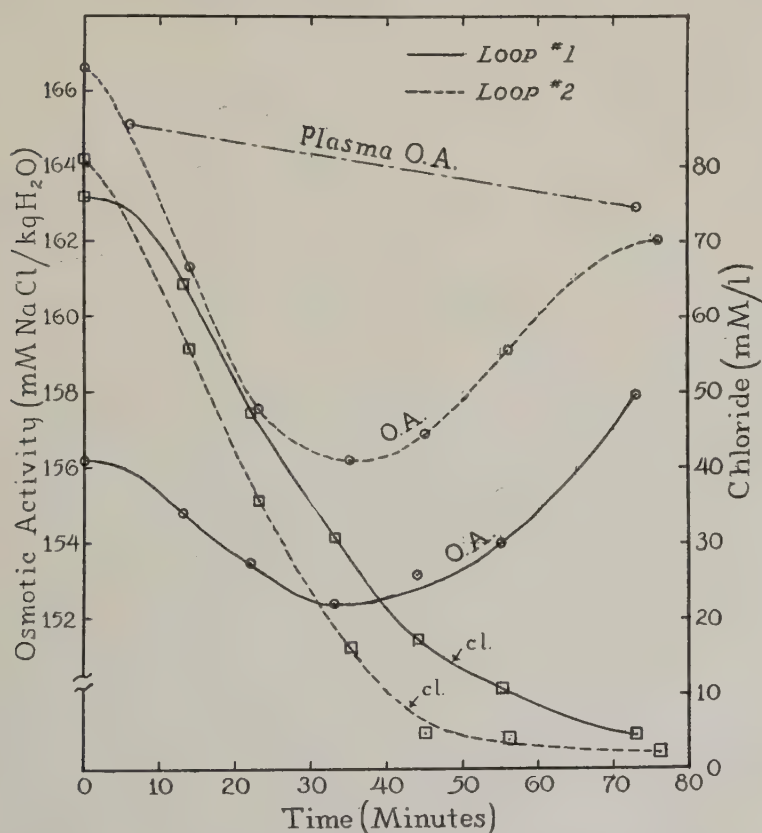


FIG. 1.

Changes in osmotic activity and in chloride concentrations of solutions of NaCl + Na₂SO₄ (in equi-osmotic proportions) when placed in isolated ileal loops.

⁶ Baldes, E. J., *J. Sc. Instruments*, 1934, **11**, 223.

water. All determinations of osmotic activity were made in a chamber containing 5% CO₂ in oxygen. Chloride was determined according to the method of Van Slyke.⁷

The animals were anesthetized and the intestinal loops prepared as described previously.⁴ Blood samples were drawn in an oiled syringe from the femoral artery, transferred out of contact of air to a test tube containing oil and the required amount of dry heparin (Connaught), the tube stoppered and the plasma separated immediately by centrifugation. Samples of intestinal fluid were drawn with needle and syringes at intervals of from 10 to 30 minutes.

Results. Five different types of experiments were conducted, using solutions of NaCl, NaCl + Na₂SO₄ in equi-osmotic proportions, Na₂SO₄, Na₂SO₄ + .001 M HgCl₂, and samples of the dog's own blood serum. In all experiments in which chloride impoverishment occurred, the osmotic activity of the intestinal fluid was decreased during absorption unless it was originally appreciably below that of the blood plasma.

The results of an experiment in which solutions containing NaCl and Na₂SO₄ were placed in isolated ileal loops are given in Fig. 1. It should be noted that during the period of maximum rate of chloride impoverishment, the osmotic activity of the intestinal fluid decreased even though it was originally below that of the blood plasma. At the end of 35 minutes, the osmotic activity of the solution in one loop of the intestine is below that of the blood plasma by an amount corresponding to a difference in osmotic pressure of 355 mm Hg.* In the second loop the difference corresponds to 230 mm Hg.

The osmotic activity of the intestinal fluid was also decreased below that of the plasma when the dogs' own serum or solutions of NaCl were placed in the loops although as a rule the changes were not as great as with solutions of NaCl + Na₂SO₄. With solutions of Na₂SO₄, the osmotic activity of the intestinal fluid approached that of the plasma at approximately the same rate whether the solutions were originally hypotonic or hypertonic. With solutions of Na₂SO₄ containing 0.001 M HgCl₂ as a poison, the osmotic activity of the intestinal fluid rose above that of the blood plasma due, apparently, to the diffusion of NaCl from the blood into the intestinal loop.

Summary. During the process of absorption of various solutions

⁷ Van Slyke, D. D., *J. Biol. Chem.*, 1923, **58**, 523.

* Calculated according to the relation used by Culbert, McCune, and Weech.⁸

⁸ Culbert, R. W., McCune, D. J., and Weech, A. A., *Ibid.*, 1937, **119**, 589.

from isolated loops of dog's ileum the osmotic activity of the loop fluid normally decreases. The total osmotic pressure may fall to a half atmosphere below that of the blood plasma, even when the major solute constituents of the fluid show changes in concentration opposite to those expected on the basis of simple diffusion. These observations appear to leave no doubt as to the occurrence of active osmotic work in intestinal absorption, but they do not permit conclusions concerning the mechanism by which it is performed.

The authors are indebted to Dr. E. J. Baldes of the Mayo Foundation of the University of Minnesota for the loan of the thermoelectric apparatus.

10725

Some Effects of Menopause Urine Extract on Sexual Organs of Immature Female Cats.

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It has been shown that menopause urine extract is capable of inducing estrus in the mature anestrus cat.¹ Mature and immature cats (ages not stated) have shown a similar reaction when FSH was administered.² Bourg³ reported that in 2 immature cats of 45 days of age and in 2 cats of 15 days of age the ovaries were capable of responding to pregnancy urine extract. Our work was undertaken to study the effect of menopause urine extract on the immature female cat of known age with reference to the general effect on the ovary and uterus.

Menopause urine (pooled samples) was acidified with glacial acetic acid and precipitated with 95% alcohol. The precipitate was washed with ether and stored as a powder. This single batch of material was used for all experiments. The powder was dissolved in distilled water, as needed, and injected subcutaneously. Mature cats re-

¹ Collings, W. D., Ph.D. Thesis Princeton Univ. Library, 1938; *Proc. Soc. Exp. Biol. and Med.*, 1939, **40**, 679.

² Foster, M. A., and Hisaw, F. L., *Anat. Rec.*, 1935, **62**, 75.

³ Bourg, R., *Compt. Rend. Soc. Biol.*, 1932, **111**, 148.

spond to a daily dosage of 20 r.u. of this hormone per kilo body weight.¹ A 20 or 25 r.u. dosage was used and a fairly comparable total dosage was administered in all cases.*

Twelve immature female cats, 6 to 13 weeks of age, were employed in these experiments, of which 4 served as controls, 3 of which were litter-mates in the groups in which they served. The 4 control animals varied in age from 6 to 12 weeks, but all exhibited a corresponding condition. The vaginal smears consisted of epithelial cells and a few leucocytes. The uterus was very small and the endometrium showed only slight glandular development (Fig. 2). The ovaries showed only small vesicular follicles (Fig. 1).

The uterine condition of the experimental animals closely paralleled the state of the ovary. Good stimulation of the ovary (Fig. 3) was accompanied by hypertrophy of the myometrium and a typical estrus endometrium with a good glandular development (Fig. 4). The presence of lutein tissue in the ovary (Fig. 5) resulted in a pseudopregnant endometrium in which the glands extended to the myometrium. The glands were lined with tall columnar epithelium and appeared to be actively secreting. Sixteen days after the last injection the endometrium was only beginning to regress from a pseudopregnant phase (Fig. 7). In animals where only one ovary responded and the stimulated ovary was removed, a rapid regression of the endometrium was observed.

Two cats (5, 22) 8 weeks of age were given a total of 260 r.u. of extract. Vaginal smears became cornified on the 12th day after the start of injections and infiltration of leucocytes resulted 4 days later. These were the only cases in which completely cornified smears occurred. Cat 5 was sacrificed but showed only a slight response with the development of a cystic follicle in 1 ovary. The other cat (22) was operated 14 days after the last injection. The right ovary and a portion of the corresponding uterine horn was removed. Although the ovary did not exhibit stimulation at this time, the regression of a stimulation of the endometrial crypt glands was observed. Five days later, a second series of injections was begun in which 220 r.u. were given in 12 days, the cat being sacrificed on the 16th day. The left ovary contained 4 follicles, one of ovulating size with what appeared to be a normal ovum (Fig. 6). No corpora lutea were present.

Two animals (20, 21), 11 weeks of age, received 215 r.u. in 10 injections. Two days later the right ovary and a portion of the

* One rat unit was the minimum amount which, when injected twice daily into 30-day-old female rats for 3 days, would produce only large follicles in 96 hours.

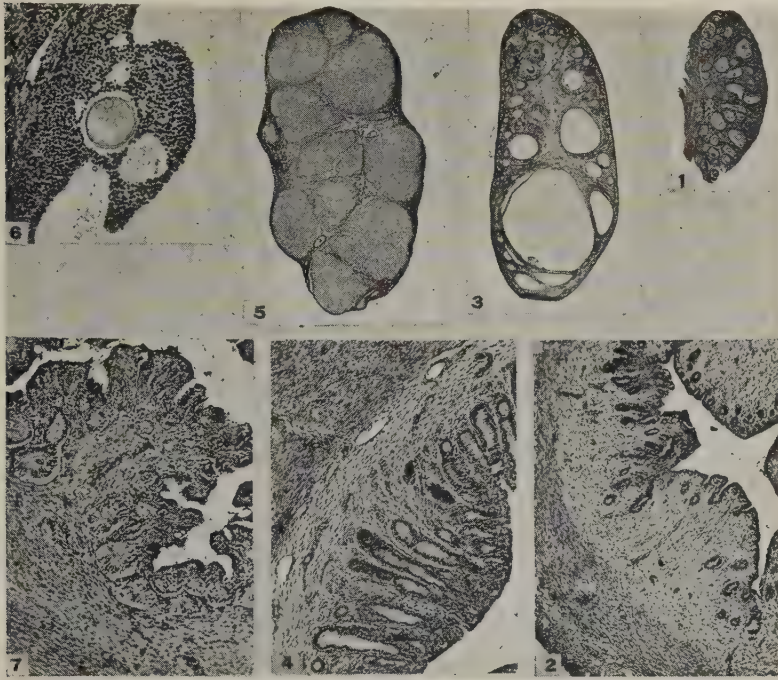


FIG. 1. Normal ovary of immature cat (No. 6). $\times 4$.

FIG. 2. Normal uterus of immature cat (No. 6). $\times 45$.

FIG. 3. Right ovary of cat No. 22G showing one large follicle containing a normal ova and several smaller follicles. $\times 4$.

FIG. 4. Uterus (right) of cat No. 22G showing endometrium of the estrus type with extensive glandular development. $\times 45$.

FIG. 5. Left ovary of cat No. 12 at the time of sacrifice showing extensive corpora lutea development. $\times 4$.

FIG. 6. Ova, apparently normal, shown in follicle of ovary in Fig. 3. $\times 55$.

FIG. 7. Uterus of cat No. 12 at the time of sacrifice. Endometrium is regressing from the pseudopregnant type. $\times 45$.

uterus was removed. Gross observation of the left ovary failed to indicate any stimulation. Cat 20 had not responded, but cat 21 had one large follicle in the right ovary and no corpora lutea. Two days later, both animals were subjected to a second series of injections and received 20 r.u. daily for 12 days and were sacrificed 4 days later. Again cat 20 failed to respond although having received a total of 455 r.u. The other cat (21) did not exhibit follicle stimulation in the left ovary, but a small amount of lutein tissue was observed. Thus, a second series of injections failed to initiate a response in a cat which had not responded to the first test. Cat 21 failed to show follicle stimulation in the left ovary as a result of further treatment although the right ovary had responded to the initial injections.

Two 7-week-old cats (12, 13) were given 25 r.u. daily for 6 days and after a lapse of 4 days were given 7 daily injections of 20 r.u. On the 4th day after the last injection, both animals were operated and the right ovary and a portion of the uterus was removed. In cat 12, the ovarian weight increased four-fold over the average control weights for one ovary. Gross observation of the left ovary revealed stimulation. The right ovary had one large follicle, but consisted mostly of lutein tissue, much of which was atretic corpora lutea. Fourteen days after the operation, the animal was sacrificed, no injections being given during this period. The left ovary contained a few follicles with thickened granulosa, but in the main was composed of corpora lutea. As many as 10 corpora were present in one section. The pseudopregnant state was maintained for a much shorter time than apparently exists in normal pseudopregnancy although the corpora lutea showed no evidence of vacuolation. The other cat (13) had one large follicle covering most of the surface of the right ovary at the time of removal, while the left ovary did not appear to be stimulated. The right ovary revealed a large cystic follicle and 2 smaller normal follicles with thickened granulosa. This animal was sacrificed 9 days later and the left ovary failed to exhibit stimulation. The markedly regressed endometrium indicated the absence of ovarian secretion. Thus, again, the right ovary had responded whereas the left ovary had not.

There was no indication that ovulation had occurred in any of the experimental animals.

One cat (23G), 6 weeks of age, was injected with a total of 160 r.u. in 9 days. This animal did not react to treatment. A 13-week-old cat (19G), which had received 220 r.u. in 12 days also failed to respond.

Summary and Conclusions. Five of 8 immature female cats, 6 to 13 weeks of age, responded to menopause urine extract with follicle stimulation although lutein tissue predominated in one case. Ovarian weight increased as much as four-fold as compared to 4 immature controls. In general, the right ovary responded to a greater extent than did the left ovary. In 2 cases, the left ovary had not responded, whereas the right ovary was stimulated. A second series of injections failed to initiate a response in one cat which had been negative to the first test and the left ovary of another cat failed to respond to further injections although the right ovary had responded to the initial treatment. This lack of uniformity in response appeared even between members of the same litter. It would appear that neither the general condition nor the age of the animal was the factor determining the response, but rather some property peculiar to the individual ovary.

A typical estrus endometrium was associated with follicle stimulation whereas a pseudopregnant development was obtained in the presence of lutein tissue in the ovary. However, regression from the pseudopregnant phase was observed 16 days after the last injection.

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Xylose as a Cataractogenic Agent.*

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Mitchell and Dodge first reported the occurrence of cataract in rats fed rations containing large quantities of lactose.¹ It appears probable that the galactose portion of the lactose molecule was the causative agent.^{2, 3, 4} It has been stated that galactose is a unique causative agent in the experimental production of lens opacities in the rat.⁵ Other carbohydrates which have been adequately investigated for possible cataractogenic activity are glucose and fructose and substances which yield only these 2 monosaccharides upon hydrolysis. We have therefore undertaken the investigation of other less common monosaccharides with respect to this property.

Rats weighing 32-47 g and 21-22 days of age were given diets consisting of 18% casein, 3% salt mixture, 2% cod liver oil, 6% butter fat, 10% dried yeast, 26% cornstarch, and 35% monosaccharide. Glucose, galactose, and xylose were used in this series. The C. P. galactose was obtained from Pfanstiehl Chemical Co. Two lots of xylose were used; the technical grade (Pfanstiehl) was used in the diets of 12 rats and a sample of C. P. xylose (Pfanstiehl) was given to 4 rats. Five litters of rats were used, 2 from a Wistar strain of albinos and 3 from a strain of black and white

* Research paper No. 520, journal series, University of Arkansas.

¹ Mitchell, H. S., and Dodge, W. M., *J. Nutrition*, 1935, **9**, 37.

² Yudkin, A. M., and Arnold, C. H., *Proc. Soc. Exp. Biol. and Med.*, 1935, **32**, 836.

³ Mitchell, H. S., *Proc. Soc. Exp. Biol. and Med.*, 1935, **32**, 971.

⁴ Day, Paul L., *J. Nutrition*, 1936, **12**, 395.

⁵ Mitchell, H. S., Cook, G. M., and Merriam, O. A., *J. Nutrition*, 1937, **13** (supplement), 18.

TABLE I.
Incidence of Cataract in Rats Fed Adequate Diets Containing 35% of Various Sugars.

Sugar fed	No. of rats	No. showing cataract	Avg time of appearance of cataract, days
Glucose	7	0	—
Xylose	16	16	4.2
Galactose	6	6	5.7

hooded rats. Some of the rats from each litter received glucose, others received galactose, and still others received xylose. Daily ophthalmoscopic examinations were made after dilating the pupil with 0.5% atropine sulfate solution. Slit-lamp examinations of the eyes were also made.

All of the rats fed either xylose or galactose developed cataract, while none of those receiving glucose developed lenticular changes. The results are presented in Table I. The time of appearance tabulated is for the earliest changes observable with the ophthalmoscope. Eight of the xylose-fed rats developed mature cataracts in from 14 to 26 days. Four of the galactose-fed animals developed mature cataracts in from 18 to 24 days. The others of both groups were killed for histologic study before the opacities were complete. In all of the animals fed xylose the first opacities were subcapsular, either in the equatorial region or in the posterior cortex. The nucleus was seldom involved. The opacities were not transitory since they persisted after the rats were returned to a normal stock diet. No appreciable difference in the response of animals to the 2 grades of xylose has been noted.

Blood sugar data will be presented in a later report. The average blood sugar level in rats receiving xylose was higher than the average in litter mates receiving glucose but lower than that in animals fed galactose. This suggests that the ability of a carbohydrate to raise the blood sugar level may not be the only factor determining its injurious action on lens tissue. Since galactose and xylose have certain configurations in common, it is conceivable that this injurious action may be a property of a number of sugars with particular configurations.

Summary. Young rats given a diet containing 35% xylose developed cataractous changes within approximately the same length of time as did litter mates receiving a similar diet containing 35% galactose. Controls receiving glucose showed no lenticular changes. It is suggested that the cataractogenic activity of sugars may be dependent upon certain molecular configurations.

Inhibitory Effect of Urine Extracts on Gastric Secretion.

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It was found by Sandweiss, Saltzstein and Farbman^{1, 2} that extracts prepared from the urine of pregnant women as well as from the urine of normal non-pregnant women exerted striking protection against Mann-Williamson ulcers when administered subcutaneously. The association of this type of experimental ulcer with an unneutralized gastric secretion naturally raised the question of what effect these extracts have on normal gastric secretion. These workers² had observed that daily subcutaneous injections in dogs of 1 or 5 cc of these extracts of urine (the daily dosage found effective in their Mann-Williamson dogs) had no effect on the acidity of the gastric secretion stimulated by a meal. In the case of pregnancy urine extracts this ineffectiveness of small subcutaneous doses was confirmed by Culmer, Atkinson and Ivy³ who further found that daily subcutaneous injections of 10 to 20 cc depressed the volume of secretion of dogs with total gastric pouches.

At the request of one of us (D. J. S.) Professor Ivy kindly tested the effect on gastric secretion of *intravenous* administration of extracts of normal female urine. In dogs with entire gastric pouches there was a marked inhibition of gastric secretion stimulated by histamine.⁴ Since then, on extending this work to extracts of urine from other sources, Ivy and his co-workers⁵ have found a gastric secretory depressant in the urine of the normal human male and of the dog. Necheles⁶ recently reported a similar extract differing from the above in that it also inhibited gastric motility.

The present paper is a report on the effect on gastric secretion of

¹ Sandweiss, D. J., Saltzstein, H. C., and Farbman, A., *Am. J. Digest. Dis.*, 1938, **5**, 24.

² Sandweiss, D. J., Saltzstein, H. C., and Farbman, A., Detroit Physiological Society, March 3, 1938; also *Am. J. Digest. Dis.*, 1939, **6**, 6.

³ Ivy, A. C., personal communication; also Culmer, C. U., Atkinson, A. J., and Ivy, A. C., *Proc. Am. Physiol. Soc.*, 51st meet., 1939, p. 56.

⁴ Ivy, A. C., personal communication.

⁵ Gray, J. C., Wieczorowski, E., and Ivy, A. C., *Proc. Am. Physiol. Soc.*, 51st meet., 1939, p. 91.

⁶ Necheles, H., personal communication.

intravenous administration of urine from normal (non-pregnant) females and from peptic ulcer patients. Such a study was believed to be of interest since it was found² that extracts of urine from ulcer patients did not have the protecting action on the Mann-Williamson ulcer that normal urine extracts had.

The procedure of Katzman and Doisy⁷ for obtaining the A. P. L. hormone in pregnancy urine (Antuitrin-S) was employed in preparing the extracts used in our study.* Assays were made on 47 vagotomized dogs under nembutal anaesthesia, the animals having been previously fasted for 24 hours. Gastric juice was obtained by fistula from the whole stomach, the pylorus and oesophagus being tied off to exclude contamination by regurgitated intestinal contents and oesophageal mucus. Gastric secretion was stimulated by hourly subcutaneous injections of histamine phosphate (0.1 mg per kilo per hour). Urine extracts were administered by femoral vein in doses of 1 mg per 5 kilo body weight.

Results. 1. The profuse secretion of acid gastric juice which is stimulated by histamine injection (see "control experiments," Fig. 1) was prevented if normal urine extract was administered before the histamine. Although hourly subcutaneous injections of histamine were repeated for as long as 6 hours, there persisted throughout this time a complete inhibition of gastric secretion. 2. When given 2 or 3 hours after the initial hourly injection of histamine, extracts of normal urine exerted a profound inhibition of the gastric secretion. This inhibitory effect commenced within 30 to 45 minutes and lasted for a period of 3 to 4 hours (Fig. 1). 3. Normal urine extracts abolished completely the secretion induced by histamine if they were administered 7 to 9 hours after the initial hourly histamine injection. However, by this time the rate of secretion in the control animals was declining so that it can not be said that a greater depressing effect was exerted when the extract was given on the 7th to 9th hour than when given earlier. 4. Extracts prepared from the urine of duodenal ulcer patients also exerted a marked depressing effect on gastric secretion (Fig. 1). 5. The principle in the normal urine responsible for depressing gastric secretion was only slightly, if at all, inactivated by heating the extract at 99°C for 30 or 60 minutes. However, heating at this temperature for 4 hours inactivated the extract completely (Fig. 1).

Conclusions. From the urine of normal individuals there can be

⁷ Katzman, P. A., and Doisy, E. A., *J. Biol. Chem.*, 1932, **98**, 745.

* The authors are greatly indebted to Dr. Oliver Kamm of Parke, Davis and Company for his kind assistance.

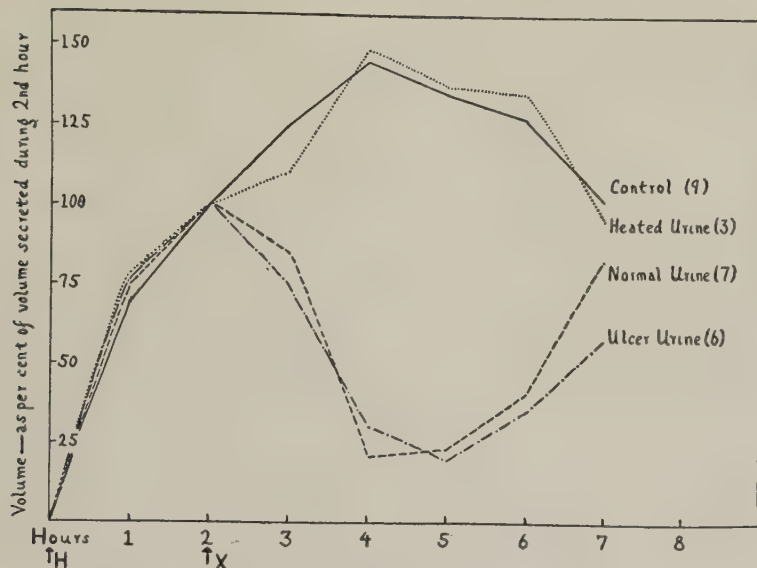


FIG. 1.

Of the 47 experiments, 25 are represented by the above series. The hourly rate of gastric secretion is expressed in terms of per cent of the volume secreted during the second hour. Histamine phosphate (0.1 mg per kilo) was given subcutaneously every hour, commencing at the point H. Urine extracts were given intravenously at the end of the second hour, at the point X. The nature of the experiment and the number of animals used are indicated.

extracted a substance which when given intravenously inhibits gastric secretion stimulated by histamine. This gastric secretory depressant is inactivated by heating for 4 hours at 99°C. When given subcutaneously, the daily amount found effective for protection against the Mann-Williamson ulcer is too small a dosage to reduce gastric secretion.² Extracts of urine from ulcer patients do not protect against the experimental ulcer but do reduce gastric secretion. From this and other data it would seem probable that the apparent beneficial effect on Mann-Williamson ulcers previously reported may be due to some factor in the urine extract other than the one which inhibits gastric secretion. We do not as yet know the nature of the substances nor what organ or mechanism of the body is responsible for their elaboration.

Supplementary Proteins and Amino Acids and Dietary Production of Fatty Livers in Mice.

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In a previous report¹ it was concluded that, whereas a high fat diet supplemented with cystine produced fatty livers in white rats, supplementary methionine exerted a lipotropic action. While the present study was devised primarily to determine the effects of high fat diets supplemented with homocystine* and cysteine, other diets were included because white mice were used and it was thus necessary to determine whether the level of the liver lipids of mice could be altered in the same manner as those previously reported for rats.

All diets contained 2% agar, 40% lard, 5% salt mixture,² 5 or 20% protein and 33 to 48% glucose (depending on the protein level). Each mouse received 100 mg dried yeast and 1 drop of cod liver oil daily. The proteins used were casein and arachin, the latter being employed because of its low methionine content. The amino acid supplements of the diet are shown in Table I. The duration of the experiments was 3 weeks and the procedures employed were those used previously in the rat experiments.¹

The table shows that methionine and cystine influence the level of liver lipids of mice as observed in rats. Increasing the casein content from 5% to 20% produces a marked drop in liver lipids, whereas a similar increase in the arachin content of the diet was ineffective. This was probably due to the low methionine content of arachin. Homocystine as a supplement behaves like cystine. This was unexpected because of the report of Beach and White³ that homocystine, like methionine, promotes growth in rats on an arachin diet. Channon and co-workers,⁴ however, stated that in one experiment conducted on the rat, homocystine showed a tendency to resemble cystine in its action and duVigneaud, at the 1939 meeting of the Federation of American Societies for Experimental

¹ Tucker, H. F., *J. Biol. Chem.*, 1937, **121**, 479.

* The homocystine was kindly supplied by Professor V. duVigneaud of Cornell University.

² Osborne, T. B., and Mendel, L. B., *J. Biol. Chem.*, 1919, **37**, 572.

³ White, A., and Beach, E. F., *J. Biol. Chem.*, 1937, **122**, 219.

⁴ Channon, H. J., Manifold, M. C., and Platt, A. P., *Chem. and Indust.*, 1938, **57**, 600.

TABLE I.
Influence of Diets on Average Total Lipids in Livers of Mice. Figures in Parentheses Are Ranges with the Individual Mice.

Diet	No. of animals	Liver		Liver lipids	
		g	% of body weight	mg	% of liver
20% Arachin	8	2.46 (1.53-3.67)	11.1 (8.3-18.1)	558 (311-919)	23 (16.7-32.9)
5% Arachin	14	1.36 (0.78-2.30)	8.5 (5.0-14.1)	322 (153-850)	22.5 (13.5-37.0)
20% Casein	8	1.18 (0.91-1.60)	6.5 (4.8-10.2)	131 (119-241)	7.2 (5.9- 9.9)
5% Casein	6	2.01 (1.63-3.12)	9.8 (8.7-14.2)	496 (380-575)	24.2 (19.7-32.5)
5% Arachin	14	2.18 (1.68-2.54)	12.8 (10.3-16.2)	748 (604-872)	34.6 (27.2-39.2)
+ .43% Cystine					
5% Arachin	9	1.47 (1.09-1.82)	7.0 (5.1-10.6)	122 (62-231)	8.5 (3.6-17.7)
+ .64% Methionine					
5% Arachin	8	1.82 (1.26-2.63)	12.2 (8.6-17.8)	658 (354-1025)	35.5 (23.6-45.6)
+ .5% Homocystine					
5% Casein	4	1.92 (1.68-2.28)	11.8 (9.6-18.1)	513 (319-619)	31.5 (29.6-37.4)
+ .5% Homocystine					
5% Arachin	10	1.91 (0.87-2.43)	12.0 (9.2-18.3)	683 (260-1025)	35.4 (29.9-42.0)
+ .7% Cysteine HCl					

Biology, reported that fatty livers were obtained when homocystine was added to the diet of white rats. It is evident from the reports on rats and our data on mice that homocystine itself is not lipotropic. Our data further show that cysteine acts like cystine and not like methionine.

Summary. Fatty livers have been produced in mice by diets which have previously been shown to produce such an effect in rats. High fat diets containing 5% casein, 5% arachin, 20% arachin or 5% arachin supplemented with cystine produced fatty livers in mice. A lipotropic effect occurred when methionine was substituted for cystine in the 5% arachin diet or when a high fat diet containing 20% casein was fed. Fatty livers were obtained when cysteine was added to the 5% arachin diet or when homocystine supplemented the 5% casein or 5% arachin diets. The effects produced when cystine or methionine supplemented the 5% arachin diet resembled those previously reported on rats when these amino acids supplemented a 5% casein diet. The results obtained with the 5% and 20% casein diets were also like those reported for rats.

Preparation of Blood Lipid Extracts Free from Non-Lipid Extractives.

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Deficiencies in Purification of Lipids by Petrol Ether. Resolution in petrol ether has been a classical procedure for analytical purification of extracted fats. Thus the blood fats extracted with Bloor's¹ efficient alcohol-ether mixture are, for certain analyses, dried and purified by resolution in petrol ether.^{1, 2, 3} The non-lipid extractives, such as urea, glucose, amino acids, and inorganic salts, dissolve in varying amounts in the alcohol-ether, but they do not by themselves dissolve in petrol ether.

It has been recognized, however, that the petrol ether solutions show higher N:P ratios than could be expected from any of the known phosphatides. Several attempts have been made to identify the extra nitrogen.^{4, 5, 6}

The present writers have been able to identify most of it as urea, determinable with urease and other urea reagents. Urea by itself is insoluble in petrol ether, but dissolves measurably in it when the blood lipids are present. Measurable amounts of amino acids, determinable by the specific amino acid carboxyl method of Van Slyke and Dillon,⁷ are also present.

On the other hand, petrol ether fails to redissolve the phosphatides completely. A fraction of them remains in the undissolved residue. It is slight in normal plasmas, but in certain pathological ones it may represent 40% of the phosphatides. It has the following properties suggestive of sphingomyelin: soluble in alcohol, insoluble in petrol ether, N/P ratio of 2, C/P ratio of about 45. All the other lipids seem to be completely redissolved by the petrol ether.

Proposed Extraction. The proteins and lipids are precipitated together by colloidal iron, and the water-soluble extractives are

¹ Bloor, W. R., *J. Biol. Chem.*, 1928, **78**, 53.

² Boyd, E. M., *J. Biol. Chem.*, 1933, **101**, 323; 1935, **110**, 61.

³ Kirk, E., Page, I. H., and Van Slyke, D. D., *J. Biol. Chem.*, 1934, **106**, 203.

⁴ Channon, H. J., and Collinson, G. A., *Biochem. J.*, 1929, **23**, 663.

⁵ Page, I. H., Pasternack, L., and Burt, M. L., *Biochem. Z.*, 1930, **223**, 445.

⁶ Van Slyke, D. D., Page, I. H., Kirk, E., and Farr, L., *Proc. Soc. Exp. Biol. and Med.*, 1935, **32**, 837.

⁷ Van Slyke, D. D., and Dillon, R. T., *Compt. rend. Lab. Carlsberg*, 1938, **22**, 480.

washed away. The lipids are then extracted by stirring up the wet precipitate with alcohol and ether.

To one volume of plasma in a centrifuge tube one adds in succession, with stirring, 15 volumes of water, 1.25 volumes of colloidal iron solution (Merck's "Dialyzed Iron" with 5% Fe_2O_3), and 0.65 volume of a 1:1 aqueous solution of $\text{MgSO}_4 \cdot 4\text{H}_2\text{O}$. The precipitate is centrifuged for 5 minutes, and is then washed by centrifugation with 15 volumes of water plus 0.65 volume of the 1:1 $\text{MgSO}_4 \cdot 4\text{H}_2\text{O}$ solution. The washing can be repeated as many times as necessary for desired completeness. The last washing can be done without adding the MgSO_4 . For routine analyses 2 washings suffice.

The washed precipitate is transferred to a volumetric flask marked to contain 10 times the volume of the plasma sample. For the transfer 4 volumes of absolute alcohol and 4 volumes of ether are used as follows. The precipitate is suspended in 2 volumes of the alcohol, and transferred as completely as possible to the flask. To finish the transfer one then uses in succession 1 volume of alcohol, 1 volume of alcohol, 2 volumes of ether, and 2 volumes of ether, finally filling to the mark with ether. The mixture is filtered.

For complete extraction of the lipids the presence of water is necessary in about the ratio of 1 volume to 6 of alcohol-ether, which is approximated by the above conditions. If a larger proportion of alcohol-ether per volume of plasma were taken, water would have to be added also in order to insure quantitative extraction of the lipids.

The clear filtrate contains all the plasma lipids, and we have not found in it any evidence of non-lipid extractives.

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Desoxycorticosterone Acetate Is Estrogenic in the Human Female.

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It has previously been shown that women, after surgical castration, excrete estrogens.¹ Furthermore, it was noted that in some

¹ Frank, R. T., Goldberger, M. A., and Salmon, U. J., *Proc. Soc. Exp. Biol. and Med.*, 1936, **33**, 615.

women, many months after removal of both ovaries, the vaginal smears² and vaginal mucosa³ do not reveal the usual regressive changes associated with estrogen deprivation. Both these observations suggested that there is some extra-ovarian source for estrogen formation in the body. The question arose as to whether the adrenal cortex may not be the source of these estrogens. To determine this point, a number of post-menopause women (with vaginal smear signs of estrogen deprivation) were given synthetic adrenal cortex hormone, in order to see if the hormone would have an estrogenic effect. The latter was evaluated on the basis of changes in the vaginal smears as previously reported with estrone and estradiol benzoate by Papanicolaou and Shorr⁴ and confirmed by Salmon and Frank.³

Kendall and his co-workers⁵ and Reichstein and Steiger^{6, 7} have isolated a number of active crystalline compounds from cortical hormone extracts. Synthesis of desoxycorticosterone acetate (21-hydroxyprogesterone), apparently the most active of the synthetic adrenal cortex steroids, was performed by Steiger and Reichstein.⁸ In the present study desoxycorticosterone* was used, since it was shown by Miescher, Fischer and Tschopp⁹ that the physiologic action of the synthetic adrenal cortex hormone was prolonged by esterification.

A group of 10 women with typical estrogen deficiency vaginal smears were injected with desoxycorticosterone acetate, 3 times weekly, in individual doses of 5 and 10 mg. The hormone was administered, intramuscularly, in concentrations of 5 mg per cc of sesame oil. Vaginal smears were taken 3 times weekly. The total doses administered varied from 50 to 230 mg over periods of 14 to 56 days.

The vaginal smears revealed signs of an estrogenic effect as early as 96 hours after the first injection of the desoxycorticosterone

² Salmon, U. J., and Frank, R. T., *Proc. Soc. Exp. Biol. and Med.*, 1936, **33**, 612.

³ Geist, S. H., and Salmon, U. J., *Am. J. Obs. and Gyn.*, in press.

⁴ Papanicolaou, G. N., and Shorr, E., *Am. J. Obs. and Gyn.*, 1936, **31**, 806.

⁵ Kendall, E. C., Mason, H. L., Hoehn, W. M., McKenzie, B. F., *Proc. Staff Meeting of Mayo Clinic*, 1937, **12**, 136.

⁶ Reichstein, T., *Helv. Chem. Acta*, 1937, **20**, 953.

⁷ Steiger, M., and Reichstein, T., *Nature*, 1938, **141**, 202.

⁸ Steiger, M., and Reichstein, T., *Nature*, 1937, **139**, 925.

⁹ Miescher, K., Fischer, W. H., and Tschopp, E., *Nature*, 1938, **142**, 435.

* For the desoxycorticosterone acetate used in this investigation, I am indebted to Dr. Erwin Schwenk of the Schering Corporation, Bloomfield, N. J., and Mr. R. Mautner of the Ciba Company, Summit, N. J.

acetate. This was manifested by the appearance of large, squamous epithelial cells and a decrease in the number of leucocytes and "atrophy" cells. All cases showed a full estrogen effect at the end of 8 days, with doses varying from 40 to 60 mg. At this time, the smears showed complete absence of leucocytes and "atrophy" cells and consisted entirely of well shaped, squamous, epithelial cells with small, deeply-staining nuclei. The changes in the smears during the period of corticosterone administration were strikingly similar to those observed after the administration of estradiol benzoate.

It is interesting to note that the patients experienced some relief of the menopause symptoms. The clinical improvement, however, was not as marked as one would expect from the cytologic changes in the smears. Comparable smear changes induced with estradiol benzoate are usually associated with much more striking relief of the symptoms. It is also worthy of note that the corticosterone injections had no appreciable effect on the blood pressure and, in the doses given, induced no signs or symptoms of virilism. This is particularly significant in view of the virilism and hypertension associated with adrenal cortex tumors and would seem to indicate that the adreno-genital syndrome is not caused by desoxycorticosterone.

A fact of considerable significance is that desoxycorticosterone, in doses of 0.1 to 0.5 mg, had no estrogenic effect in rats. Does this signify that desoxycorticosterone is converted in the human into an estrogen and excreted as such?

Summary and Conclusions. Typical estrogenic effects were produced with synthetic adrenal cortex hormone (desoxycorticosterone acetate) in the vaginal smears of post-menopause women. On the basis of these observations, it is suggested that the estrogens excreted after the menopause or after surgical castration probably have their origin in the adrenal cortex. This may explain the persistence of estrogen effects in the vaginal mucosa and vaginal smears after surgical castration and may, possibly, also account for individual variations in the severity of symptoms experienced at the menopause or after surgical castration.

Stimulation of Adrenal Cortex of Pigeons by Ant. Pituitary Hormones and by Their Secondary Products.

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Earlier work indicated that intact pigeons are very resistant to the toxic action of insulin and that under heavy dosage with insulin a rapid increase of adrenal cortical tissue occurs.^{1, 2} It is well known that thyroxine induces cortical hypertrophy in various mammals where this action is believed to be mediated through the anterior pituitary gland. The ability of both male and female sex hormone to stimulate one or another type of cortical cell has been reported.^{3, 4, 5} A variety of toxic substances and conditions of stress also give rise to cortical hyperplasia.^{6, 7} These considerations, along with relatively difficult or insufficiently tried methods for the assay of "adrenotropin" (or corticotropin), still lead some investigators to reserve judgment concerning the conclusiveness of present evidence for the production of a distinct (and single) adrenotropic hormone by the hypophysis. Gonads and thyroids do not seem similarly susceptible of having their size and function *augmented* by such a variety of agents. Though it is established that the pituitary supports cortical function in one or another way, and though the earlier evidence for the existence of adrenotropin^{8, 9} has since been notably supported by proofs that prolactin does not sustain or repair the adrenal cortex,^{10, 11, 12} further data concerning the basis or bases of cortical repair and hypertrophy seem useful.

In a more comprehensive study we are trying to identify the cytological criteria of activity and rest in individual cells of the

¹ Riddle, O., Honeywell, H. E., and Fisher, W. S., *Am. J. Physiol.*, 1924, **67**, 333.

² Poll, H., *Med. Klin.*, 1925, **46**, 1.

³ Leiby, G. M., *Proc. Soc. Exp. Biol. and Med.*, 1933, **31**, 14.

⁴ Poll, H., *Dtsch. Med. Wschr.*, 1933, **59**, 567.

⁵ Anderson, D. H., *J. Physiol.*, 1934, **83**, 15.

⁶ Selye, H., *Endocrinology*, 1937, **21**, 169.

⁷ Higgins, G. M., and Ingle, D. J., *Endocrinology*, 1938, **23**, 424.

⁸ Collip, J. B., Andersen, E. M., and Thomson, D. L., *Lancet*, 1933, Aug. 12, 347.

⁹ Anselmino, K. J., Hoffmann, F., and Herold, L., *Klin. Wschr.*, 1933, 1944.

¹⁰ Lyons, W. R., *Proc. Soc. Exp. Biol. and Med.*, 1937, **35**, 645.

¹¹ Riddle, O., *Cold Spring Harbor Symp. Quant. Biol.*, 1937, **5**, 362.

¹² Nelson, W. O., and Tobin, C. E., *Anat. Rec. (Abst.)*, 1937, **70**, 64.

suprarenal cortex of the pigeon, and to identify those hormones which increase the number and secretory activity of these cells. Results of that study will be published elsewhere, but in the present paper we unite certain determinations made in that investigation with another series of studies (assays) showing the character (hormonal content) of a variety of extracts which did, and also others which did not, stimulate cortical tissue in the pigeon. In effect, we here bring together the results of 3 methods of assay of "adrenotropin." In the first we use increased weight of the whole adrenal of normal pigeons; and, alternatively, support (or increase) of adrenal weight in the hypophysectomized pigeon (during 10 days following the operation). In the second we utilize the cytological evidence of stimulation or non-stimulation in these pigeon adrenals. Only White Carneau pigeons 2.5 mo. old (1.9 mo. after hatching) were employed. As a third method of assay we use the method of Moon¹³ (20 mg/day for 3 days in 21-day rats), and the percentage increase in adrenal weight is used directly in our tabulation. In addition to these assays for adrenotropin all the pituitary preparations were assayed on immature doves and pigeons for prolactin, FSH and thyrotropin. Values for the 2 latter hormones are expressed in terms of percentage increase produced in testes and thyroids (over their control weights) per mg of the preparation used; in some cases (parentheses enclose these items) the assays were inadequate for determination of true values. The results are shown in Table I.

In general the adrenals of the pigeon show a rather limited capacity for enlargement by hormones in the dosages here used; but gravimetric increases exceeding 10% are here generally significant because of the uniformity of adrenal size (barring temperature effects) in these birds. Further, the cytological criteria of the presence or absence of stimulation were unequivocal except in the case of 2 groups (parentheses there used) in which the adrenals of some but not of all the tested individuals were stimulated. In both of those groups adrenal weight had been increased (normals) or partly maintained and stimulated (hypophysectomized), thus showing essential agreement for these 2 types of assay. Indeed, these 2 methods of assay gave equivalent results in all cases, unless a possible enlargement (12%) of the adrenals of normal birds under thyroxine with no cytological evidence of stimulation constitutes an exception. Moreover, these 2 methods of assay are in

¹³ MOON, H. D., PROC. SOC. EXP. BIOL. AND MED., 1937, **35**, 649.

TABLE I.
Effects on Suprarenal Weight and Activity in Young (1.9 mo.) Carnean Pigeons of Various Anterior Pituitary (Also Thyroxine and Estrone) Preparations Otherwise Assayed for Their Hormonal Content.

Assays of preparations used														
Pigeons used	Preparation injected	No. of birds	Dosage		Change in body wt during dosage, %	Effect on adrenals	Adrenotropin % increase in wt (rat)	Prolactin No. units per mg	FSH % increase in testis wt per mg	Thyrotropin % increase in thyroid wt per mg	Source of material injected			
			No. of days	Mg per day										
Normal	—	50	10	—	+3	—	—	—	—	—	Beef			
	669	3	10	10	+17	+	56	7	24	1.2	"			
	682	3	5	5	-10	+	74	.7	(0)	(.4)	"			
	680	10	10	3.3	+18	(+)	5	11	0	0.5	"			
	643	3	5	5	-9	+	10	.0	11	1.1	Pork			
	632	2	10	25	-18	+	66	.0	24	.9	Beef			
	Intermedin	8	10	1 cc	+4	—	0	.0	0	(0)	" (?)			
	Gamone	3	9	1.5	-21	+	71	.0	(2)	0	Human			
	Thyroxine	5	10	0.05	-3	—	252	.0	0	0	Synthetic			
	Estrone ³	5	16	(2)	-15	+	354	.0	0	(0)	"			
Hypophysectomized	—	24	10	—	-17	—	—	—	—	—	Beef			
	Muscle ext.	7	10	10	-245	—	8	.0	0	0	Sheep			
	582	12	10	10	+15	+	81	1	14	1.0	Beef			
	437H	6	10	3.1	+10	+	98	5.5	0	0	"			
	394H	8	10	5	-225	—	0	8	0	0	"			
	626	5	10	12	+17	—	25	6	(1)	1.1	"			
	632	10	10	2	-9	—	66	.0	24	.9	"			
	600	6	10	10	-13	+	42	.0	14	.5	"			
	Gonadogen ⁷	10	10	2 u	-15	—	?	.0	(30)	0	Horse			
	Gamone	8	10	3	-19	+	71	.0	(2)	0	Human			
Prolan	10	10	1	-20	—	8	.0	(6)	0	"				
Thyroxine+ ⁸	10	10	0.05	-21	(+)	252	.0	0	decr.	Synthetic				

* Fasted during last 48 hours.

1 As assayed at one-fourth usual level (15 mg instead of 60 mg).

2 Assayed at 0.05 mg/day thyroxine.

3 Pellet implant in addition to injection.

4 Assayed at 0.1 mg/day.

5 Fasted 10 days.

6 Glands not completely dissected.

7 A preparation of pregnant mare serum.

8 Glands not completely dissected.

very substantial agreement with the method of Moon. We regard this common response of cortical tissue of rat and bird to a common component(s) of the anterior pituitary as a significant new fact. Among the true pituitary preparations discrepancy is observed only with No. 680; this preparation increased adrenal weights in normal pigeons and gave cytological stimulation in most of them, though it failed (apparently a 5% increase, but muscle extract gave an increase of 8%) to indicate the presence of adrenotropin by the Moon test.

Seven of the preparations used contained prolactin, but it is clear that their support of the adrenal was not at all related to the amount of prolactin injected. Birds dosed with No. 437H received much less prolactin than those treated with No. 626, but the three types of assay show that the adrenals of rats and birds were supported far better by No. 437H. It is to be noted that No. 437H tested free of FSH and thyrotropin. Gonadogen, rich in FSH and effective in maintaining the gonads of hypophysectomized pigeons, showed no ability to sustain or stimulate the adrenals of such birds. Preparations Nos. 600, 632, 643 contained no prolactin but, when given at moderate or high dose levels, they all induced marked cortical stimulation. Intermedin showed no ability to stimulate the adrenals of either rat or pigeon.

Gamone (from post-menopausal urine) produced peculiar and notable effects. This preparation stimulates the gonads of birds much less than those of rats; but at least in immature individuals of these two species it stimulates the bird adrenal far more than that of the rat; here the Moon test and the two pigeon tests give unlike or contrary results. It is further notable that under gamone dosage both medulla and cortex of the bird suprarenal are stimulated. When given at a high dosage level estrone clearly stimulates the adrenals of normal immature pigeons (and rats). The apparent ability of thyroxine (plus supplements) to stimulate and largely sustain the adrenals of hypophysectomized pigeons further suggests that similar actions of extra-pituitary hormones on cortical tissue have not been sufficiently explored.*

Summary. Increase of weight in the adrenals of the normal immature (1.9 mo.) pigeon, and degree of maintenance of adrenal

* Gamone used in this study was obtained through the courtesy of E. R. Squibb and Son; intermedin, from I. G. Farbenindustrie, through the Winthrop Chemical Co.; estrone, from the Schering Corporation; gonadogen, from the Upjohn Co. The various anterior pituitary preparations were made by Dr. R. W. Bates in this laboratory.

weight during 10 days following hypophysectomy in the pigeon, and also cytological evidence of stimulation in their cortical cells, are found to agree satisfactorily with Moon's method of assay of adrenocorticotropin in 21-day rats. As tested by these 3 methods the ability of pituitary extracts to stimulate cortical tissue is independent of their prolactin, FSH and thyrotropin potencies. Gamone stimulates both cortical and medullary tissue in both normal and hypophysectomized pigeons. Estrone stimulates cortical tissue at least in intact birds. Thyroxine, plus vitamin supplements, gave evidence of ability to stimulate cortical tissue in hypophysectomized pigeons.

10732

**Metabolic Interdependence of Vitamin B₁ and Manganese.
Reciprocal Neutralization of Their Toxic Effects.***

DAVID PERLA AND MARTA SANDBERG.

From the Laboratory Division, Montefiore Hospital, New York City.

We observed that rats fed our standard adequate, varied diet, supplemented with 100 gamma of vitamin B₁ daily, either in the form of yeast, or as synthetic vitamin B₁ (parenterally administered), demonstrated after one generation interference with lactation, loss of the maternal instinct, cannibalism and progressive loss of fertility.¹ With reduction in the amount of vitamin B₁ to 40 gamma or the elimination of the supplements of vitamin B₁ for short periods, normal lactation and normal interest in the young was restored. When the vitamin B₁ content was again increased the same toxic effects were observed.† Further study completely confirmed our earlier findings. With daily supplements of 60 gamma of vitamin B₁, progressive decrease in fertility also occurred, with a moderate incidence of loss of litters due to cannibalism. After four generations breeding decreased.

* A preliminary note of this work appeared in *Science*, 1939, **89**, 2302. Read before the American Society of Experimental Pathology, April, 1939, at Toronto.

¹ Perla, D., *Proc. Soc. Exp. Biol. and Med.*, 1937, **37**, 169.

† Interference with lactation and reproduction could not have been due to the absence of vitamins L₁ and L₂ recently postulated by Nakahara Inukai and Ugami (*Science*, 1938, **87**, 372) since yeast is a rich source of these factors.

The data of these earlier preliminary experiments are given in detail in Table I.

Our normal diet consists of the following: 15 g per rat per day of a basic mixture of hominy 100 parts, rolled oats, 25 parts, fine meat and bone 25 parts, salt 1 1-2 parts and dried skimmed milk 16 parts, to which are added a few drops of cod liver oil, 0.3 mg wheat germ and 0.3 g of crude Fleischmann's brewer's yeast per rat. The yeast product contained 6 I.U. of vitamin B₁ per gram. This is equivalent to about 2 to 3 I.U. (7 gamma) per rat per day. In addition the animals received fresh greens twice a week and fresh whole milk daily. On this complete diet it has been our experience that the rats maintain a good growth curve, reproduce well and rear their young without loss of any members of their litters.

In view of the fact that Williams² stated that as much as from 160 to 1000 gamma of vitamin B₁ daily could be given without any toxic effects when rats were fed a Sherman breeding diet (one-third whole milk and two-thirds whole wheat), it seemed probable to us that interference with some other factor in the diet might have induced the manifestations observed in our experiments.

It is known that deficiency of manganese in the diet presents similarly toxic effects on the maternal instinct and reproduction.³ It was reasoned that perhaps manganese is essential as an oxidative catalyst in the utilization of vitamin B₁ in the tissues. If this were so the available manganese in the tissues might be exhausted by an excess of vitamin B₁ and manifestations would occur analogous to those observed in manganese deficiency. Furthermore, it was observed by Hamamoto⁴ that large amounts of both vitamin B₁ and manganese are found together in nature in such sources as wheat products and the like.[‡]

To test our hypothesis we added small amounts of manganese (2 mg per rat per day as MnCl₂) to the diet. Rats which had shown loss of maternal instinct and cannibalism now bred and raised normal litters (Table I). The studies were then extended. Rats were raised on the normal diet and given parenterally 400 gamma of vitamin B₁ daily. Others were given the same diet and vitamin B₁ but the diet was supplemented with 2 mg of manganese as MnCl₂ per day per rat. The results completely confirmed our hypothesis. In those receiving the vitamin B₁ alone, cannibalism

² Williams, R. R., and Spies, T. D., *Vitamin B₁ and Its Use in Medicine*, Macmillan Co., New York City, 1938, p. 286.

³ Orent, E. R., and McCollum, E. V., *J. Biol. Chem.*, 1931, **92**, 651.

⁴ Hamamoto, E., *Orient. J. Dis. Infants*, 1935, **18**, 21, 57.

[‡] He observed a decrease in manganese in the tissues of beri-beri birds.

TABLE I.
Toxic Effects of Excess of Vitamin B₁ on Maternal Instinct and Reproduction.

Group	Yeast Excess										Betaxin Excess										Controls										
	100					100					100					100					Normal Diet Alone (7 gamma B ₁)										
Quantity of B ₁ (gamma)	100	100	50	50	0	100	+	100	+	MN	100	100	40	0	+	50	50	100	100	+	MN	100	100	+	MN	100	100	+	MN		
Generation	P	6w	F ₁	F ₂	6w	P	6w	F ₁	F ₂	6w	P	6w	F ₁	F ₂	6w	P	6w	F ₁	F ₂	6w	P	6w	F ₁	F ₂	6w	P	6w	F ₁	F ₂	6w	
No. females breeding	8	7	13	13	13	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	
No. litters	7	6	3	3	5	2	3	1	2	3	7	3	2	6	3	4	3	17	16	18	15	15	19	19	19	19	19	19	19	19	19
No. offspring	54	32	21	32	48	12	19	8	15	39	21	12	43	20	20	29	29	139	128	168	118	150	150	150	150	150	150	150	150	150	150
No. litters abandoned or eaten	2	6	0	3	1	8	0	0	2	4	2	2	4	0	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
No. offspring abandoned or eaten	16	30	0	30	10	2	0	0	12	10	13	12	21	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

TABLE II.

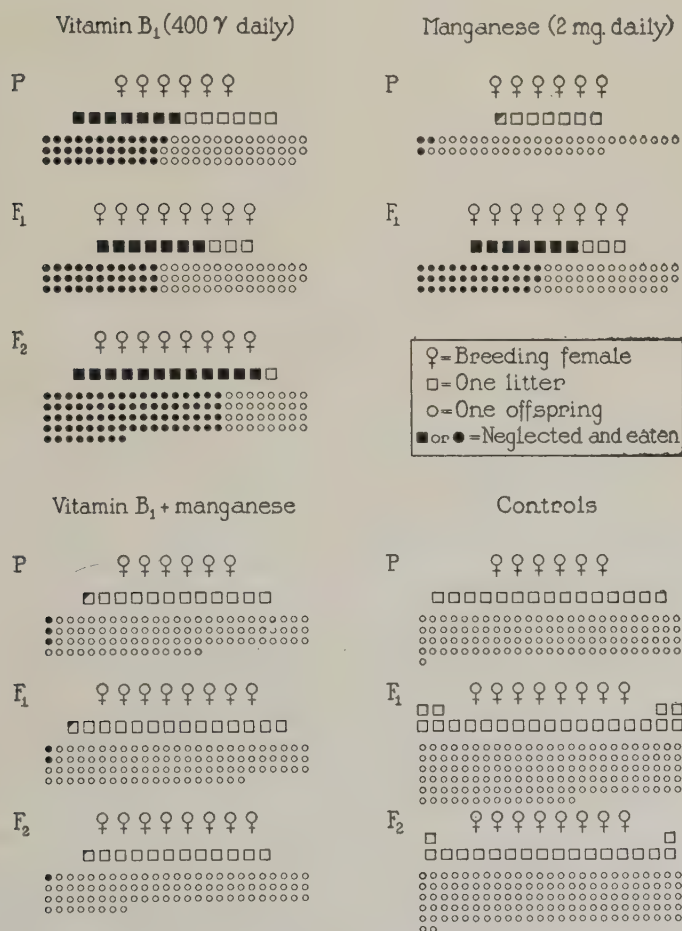
The Preventive Action of Supplements of Manganese to the Diet on the Effects of an Excess of Vitamin B₁ in Rats Fed Complete Diets.*

Group	Betaxin (400 gamma daily)				Betaxin (400 gamma) + MN (2 mg daily)				MN (2 mg daily)				Controls			
	P	F ₁	F ₂	Total	P	F ₁	F ₂	Total	P	F ₁	F ₂	Total	P	F ₁	F ₂	Total
Generation	6	8	8	22	6	8	8	22	6	8	8	22	6	8	8	22
Total No. females breeding	13	10	13	36	13	14	18	45	7	10	17	34	15	21	18	54
Total No. litters	74	74	108	256	89	94	90	273	43	74	117	234	105	140	127	372
Total No. offspring	7	7	12	26	0.3	0.2	0.1	0.6	0.3	7	7.3	14.6	0	0	0	0
No. litters neglected or eaten	54%	70%	92%	72%	2%	1%	1.8%	1.3%	4%	70%	43%	35%	0	0	0	0
Total No. offspring neglected or eaten	34	33	76	143	3	2	1	6	2	33	35	70	0	0	0	0
	46%	44%	70%	56%	3%	2%	1%	2%	4%	44%	30%	20%	0	0	0	0

* The vitamin B₁ was given parenterally from the age of 3 weeks in amounts of 400 gamma per day but the young in each generation were not disturbed for 2 to 3 weeks. Cannibalism and deaths from neglect occurred in more than one litter in any given female in 11 instances. It frequently occurred in the 2d or 3d litter and not in the first. In only 2 instances did it occur only in a first litter. In all cases the loss of young occurred in the first 4 or 5 days.

and interference with lactation occurred in a high percentage in the P, F₁ and F₂ generation in successive litters. In those receiving supplements of manganese in the diet, none of these toxic symptoms was apparent. Normal lactation and the normal maternal instinct were preserved (Table II).

Neutralization of toxic effects of an excess of either vitamin B₁ or manganese by appropriate ratio of manganese to vitamin B₁



All rats received a complete normal diet containing a minimum of 2 to 3 I.U. of vitamin B₁ (77)

FIG. 1.

Breeding chart illustrating the neutralization of the toxic effects of an excess of either vitamin B₁ or manganese by appropriate ratio of manganese to vitamin B₁.

It is extremely significant that the animals receiving the normal diet to which was added a supplement of manganese alone in amounts of 2 mg a day, likewise showed a disturbance in lactation and in maternal instinct, which was slight in the first generation (P), but pronounced in the second generation. In spite of this fact, the rats receiving both excess of vitamin B₁ and excess of manganese (each in themselves capable of inducing these toxic effects) in practically every instance reared their young normally.

In work now in progress it has been found that manganese in amounts of about $\frac{1}{2}$ mg a day per rat is more effective in neutralizing the toxic effects of an excess of vitamin B₁ (400 gamma), and in itself is less toxic than 2 mg.

These results demonstrate that manganese is essential in the utilization of vitamin B₁ in the tissues and is intimately bound up with the rôle of vitamin B₁ in the physiology of the organism. It also suggests that variations in certain constituents of the diet, such as manganese may greatly affect the vitamin B₁ requirement. With the use of large amounts of vitamin B₁ in therapy, an adequate supply of manganese must also be made available.

Our experiments further suggest that in the presence of an excess of manganese, a greater quantity of vitamin B₁ is essential. Perhaps the vitamin B₁ in the diet is rapidly exhausted under these conditions and insufficient quantities are available for normal lactation. In any case, apparently, the toxic manifestations observed with an excess of vitamin B₁ are the expression of an exhaustion of available manganese stores in the body, and the symptoms are those of insufficiency of manganese.

Summary and Conclusions. In rats fed normal adequate diets an excess of vitamin B₁ in amounts exceeding 30 or 40 times the minimal requirement results in an interference with the capacity of the mother to rear her young and with the nursing instinct. With an excess of 400 gamma this manifestation was pronounced in the parent generation but became progressively worse in the F₁ and F₂ generation. The young were neglected and eaten in over 90% of the litters in the F₂ generation.

The toxic manifestations of an excess of vitamin B₁ were found to be dependent on the ratio of manganese to vitamin B₁ in the diet. The addition of manganese to the diet in amounts of 2 mg per rat per day completely neutralized the unfavorable effects of the excess of vitamin B₁ (400 gamma daily). Practically no interference with lactation or rearing of the young was observed in animals which received both the excess of vitamin B₁ and manganese as ad-

dition to the normal diet during 3 successive generations. Apparently manganese in amounts of 1/2 mg is even more effective.

Supplements of manganese alone in amounts of 2 mg a day result in interference with lactation and cannibalism, particularly marked after one generation.

It is inferred that manganese acts as an essential catalyst in oxidative processes in which vitamin B₁ is concerned. The vitamin B₁ requirement of an animal varies with the manganese content in its diet.

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Note on the Action of X-rays on Goldfish (*Carassius auratus*).

FRIEDRICH ELLINGER. (Introduced by Marta Sandberg.)

From the Radiotherapy Department, Montefiore Hospital, New York City.

The following experiments were undertaken in order to study the suitability of goldfish for the problems of experimental radiotherapy. Goldfish have already been used successfully in experimental pharmacology.

A total of 408 commercial goldfish (96 of which were used as controls) were divided into series of 12 for each exposure. After irradiation each series or controls were placed in an aquarium containing 7000 cc of water at a temperature of 19° to 22°C for observation.

The radiation factors were: 200 KV, 30 MA, no filter, HVL 6 mm Al, target distance 50 cm, field 15 x 15 cm, intensity 230 r/min. The fishes were irradiated in an open Petri dish, 15 cm in diameter, suspended between 2 layers of gauze, flush with the surface of the water in a water phantom. This was 32 x 32 x 32 cm, thus permitting the maximum back scatter (Quimby and coworkers¹). Ionization measurements with a Victoreen chamber showed an increase in back scatter from the Petri dish of 3%.

The entire dose was given in one session and varied from 500 to 10,000 r without back scatter. When a dose of 1500 r up to 10,000 r was administered, all goldfish died 10 to 18, on an average 14 days after treatment, while nearly 100% of the controls remained alive

¹ Quimby, E. H., Marinelli, L. D., and Farrow, J. H., *Am. J. Roentgenol.*, 1938; **39**, 799.

at this period. After a dose of 1000 r only about 50% of the fishes were dead after a like period. The first intimation of a lethal effect was already noted after 500 r.

For about a week after irradiation the appearance of the fish did not change (latent period). On the sixth to ninth day, however, a brownish black pigmentation appeared on each side of the trunk. This was more marked when high doses were applied or where the fish's dorsal fin showed some black pigment. Fig. 1* illustrates this pigmentation 22 days after 1000 r (upper fish). Fig. 2* shows spread of astrospherical chromatophores. This phenomenon seems to be very interesting in connection with the results of Parker² con-



FIG. 1.

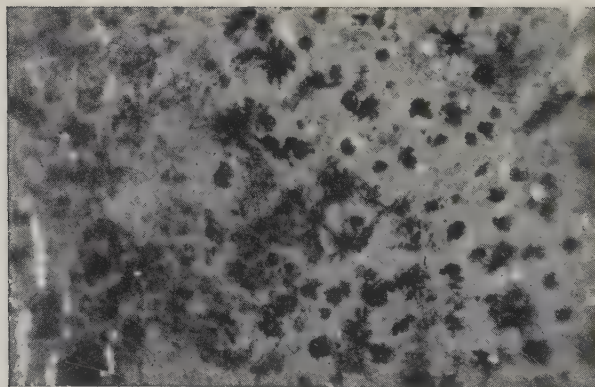


FIG. 2.
(Enlarged 80 \times .)

* I am indebted to Dr. E. Baender for the photographs.

² Parker, G. H., *Cold Spring Harbor Symposia on Quantitative Biology*, 1936, 4, 358.

cerning the neuro-humeral activation of chromatophores as well as in relation to X-ray pigmentation of mammals and human beings. (Ellinger³).

Experiments carried out together with Dr. M. Sandberg have shown that while the red pigment (Astacene according to Lederer⁴) can be easily extracted by acetone, the X-ray pigmentation still remains. With the appearance of the pigmentation the otherwise very fast moving fishes become less active. They come to the surface and die exhibiting dyspnoea.

In accordance with these observations, autopsy shows marked shrinkage and atrophy of all lymphoid tissue and pycnosis of surviving lymphocytes with increase in the amount of fibrous tissue in the lymph-nodes. In the spleen there is complete disappearance of lymphoid elements, marked proliferation of macrophages containing blood pigment (hemosiderin).†

Further observation has shown that in the range of 1: 4.5 cc the volume of the fish does not influence the lethal effect.

The similarity of the response of goldfish and mammals to X-ray, the relatively low lethal dose, as well as the possibility of getting small goldfish the year round seem to support the belief that goldfish may be used in experimental radiotherapy. The possibility of irradiating a considerable number of fishes at the same time also meets one of the prerequisites of experimental radiotherapy (Ellinger, l.c., p. 227). The fact that water, the most commonly used phantom material in depth dose measurement, is the natural medium of fish, suggests that fish may be suitable for biological depth dose measurement. Experiments along this line are in progress.

³ Ellinger, F., *Die biologischen Grundlagen der Strahlenbehandlung*, Berlin, 1935.

⁴ Lederer, E., *C. r. Soc. Biol., Paris*, 1935, **118**, 542.

† I am indebted to Dr. D. Perla for the microscopical diagnosis.

Quantitative Results of Ovariectomy in Immature and Adult Albino Rats.

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The effects of ovariectomy in young animals depend to a large extent upon the time elapsing between operation and autopsy. When ovariectomy was performed at 26 days and autopsy at 184 days (6 months), there were certain definite changes. Freudenberger and Billeter¹ reported those changes as follows: significantly heavier weights for the body, head, integument, humerus, thymus, and stomach. The suprarenal glands and uterus were definitely lighter. In addition, the tail length, and the weights of the heart, lungs and alimentary group were considered to be nearly significantly greater. There were no quantitative changes in the lengths of the body, femur, and humerus or in the weights of the femur, hypophysis, brain, spinal cord, eyeballs, submaxillary glands, intestines, liver, kidneys, and spleen.

As a result of the above experiment, we became interested in determining whether or not there might be other changes in animals somewhat older at time of autopsy (273 days). We also wished to ascertain whether ovariectomy in adult rats (177 days) would produce different results than those in pre-puberally operated animals. We decided to autopsy all of the animals at an arbitrarily set age of 273 days (9 months).

All materials, methods, and procedures were identical to those previously used and reported (Freudenberger and Hashimoto²). Reference to individual reports in the literature would entail the use of too great an amount of space for the present paper. We refer the reader to Hatai,³ Livingston,⁴ Andersen and Kennedy,⁵ and Freudenberger and Billeter¹ for more complete resumé.

Experimental Observations and Conclusions: One group of 10 female Wistar albino rats were oöphorectomized at 25 days of age. Twelve littermate controls were used. A second group of 25

¹ Freudenberger, C. B., and Billeter, O. A., *Endocrinology*, 1935, **19**, 347.

² Freudenberger, C. B., and Hashimoto, E. I., *Am. J. Anat.*, 1937, **62**, 93.

³ Hatai, S., *J. Exp. Zool.*, 1915, **18**, 1.

⁴ Livingston, A. E., *Am. J. Physiol.*, 1916, **40**, 153.

⁵ Andersen, D. H., and Kennedy, H. S., *J. Physiol.*, 1933, **79**, 1.

animals were spayed at 177 days. Twenty-five control litter-mates were employed. Both series were autopsied at 273 days (9 months).

There was a close parallel between the results of these series in spite of the fact that there was purposely, a great dissimilarity in the age at operation. In both, the spayed animals showed significantly greater measurements in body weight, integument, thymus, and stomach. The weights of the suprarenals and uterus were smaller in test animals. Negative results were found in the measurements of the body length, femur length, femur weight, humerus length, humerus weight, brain, spinal cord, hypophysis, thyroid, kidneys, and liver. Variations occurred between the 2 groups. The head weight, tail length, eyeballs, alimentary group, intestines, and spleen were significantly greater only in the test animals of the 177-273 day group. On the other hand, the heart and lungs of the younger spayed animals (25-273 days) were significantly heavier, whereas these measurements in the older group were unaffected.

Further analysis showed that the head weight of the younger test rats was nearly significantly heavier and the liver was lighter. In

TABLE I.
Average Measurements,* Differences, Values for P, and Percentages.
(25-273-day ovariectomized and control rats.)

	Control	Test	Difference	P	%
Body weight	224.66	256.2	31.54	.01	114.03
Head "	19.95	21.15	1.20	.062	105.97
Integument	34.91	41.64	6.73	.03	119.25
Body length	20.5	20.83	.33	.18	101.6
Tail "	20.08	20.59	.51	.15	102.52
Femur "	3.344	3.392	.048	.19	101.43
Femur weight	.7263	.7554	.0291	.24	103.99
Humerus length	2.582	2.623	.041	.09	101.56
Humerus weight	.3403	.3501	.0098	.30	102.86
Brain	1.9209	1.8756	.0453	.16	97.64
Spinal cord	.5816	.5899	.0083	.60	101.39
Eyeballs	.3098	.3093	.0005	.85	99.82
Hypophysis	.01422	.01325	.00097	.20	93.14
Thyroid	.01693	.01664	.00029	.79	98.26
Suprarenals	.0527	.03451	.01828	.01—	65.24
Thymus	.19165	.34707	.15542	.01—	181.09
Alimentary group	18.01	17.64	.37	.77	97.90
Stomach	1.0265	1.1976	.1711	.01—	116.67
Intestines	4.4719	4.3361	.1358	.67	96.96
Submaxillary glands	.4370	.4285	.0085	.73	98.04
Kidneys	1.8302	1.7423	.0879	.25	95.20
Uterus	.7249	.0315	.6934	.01—	4.34
Heart	.9210	1.0361	.1151	.017	112.49
Lungs	1.5692	2.2782	.7090	.022	138.81
Liver	8.8151	7.7998	1.0153	.070	88.48
Spleen	.4757	.5079	.0322	.20	106.78

* Measurements in grams and centimeters.

532 OVARECTOMY IN IMMATURE AND ADULT ALBINO RATS

TABLE II.
Average Measurements,* Differences, Significance Ratios, and Percentages.
(177-273-day ovariectomized and control rats.)

	Control	Test	Difference	Sig. Ratio	%
Body weight	222.0	252.8	30.8	6.11	113.87
Head "	19.76	21.18	1.42	5.24	107.18
Integument	32.56	37.95	5.39	6.10	116.55
Body length	21.16	21.44	.28	2.23	101.32
Tail "	19.49	20.08	.59	3.62	103.02
Femur "	3.37	3.43	.06	2.74	101.78
Femur weight	.6661	.6911	.0250	2.07	103.75
Humerus length	2.61	2.64	.02	1.40	101.14
Humerus weight	.3043	.3178	.0135	2.76	104.43
Brain	1.87413	1.87412	.00001	.0006	99.99
Spinal cord	.6206	.6195	.0011	.202	99.82
Eyeballs	.3039	.3101	.0062	3.75	102.04
Hypophysis	.01381	.01323	.00058	1.40	95.80
Thyroid	.01803	.01800	.00003	.006	99.83
Suprarenals	.0463	.0346	.0117	11.12	74.73
Thymus	.2296	.4208	.1912	11.73	183.27
Alimentary group	17.29	20.15	2.86	5.39	116.54
Stomach	.9543	1.0829	.1286	7.65	113.47
Intestines	4.5499	4.9127	.3628	3.26	107.97
Submaxillary glands	.4488	.4626	.0138	1.26	103.07
Kidneys	1.7817	1.7253	.0564	1.64	96.83
Uterus	.6666	.1125	.5541	29.23	16.87
Heart	.9247	.9803	.0556	2.54	106.01
Lungs	1.7765	1.8883	.1118	.94	106.29
Liver	8.1146	7.9652	.1494	.64	98.15
Spleen	.4411	.5170	.0759	6.11	117.20

* Measurements in grams and centimeters.

the older animals, the femur length, humerus weight, and heart weight were nearly significantly greater in the spayed animals.

A comparison of the results obtained from these 2 groups with data on younger animals (26-184 days), led to the belief that the majority of the changes due to ovariectomy occurred relatively early in the post-operative interval. There were relatively few significant changes in groups 5 months or more post-operatively. Operations in adult animals brought about changes quite similar to those produced in prepuberal operations, although quantitatively more organs were affected.

10735 P

Production of Pyrogen in Gum Acacia by Bacteria.

CO TUI, M. H. SCHRIFT AND W. F. RUGGIERO.

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The occurrence of a "reaction" consisting of fever and chills (shivering) following the intravenous use of acacia is one of the major deterrents to its widespread clinical use. There are two schools of thought on the cause of this "reaction". One school,¹⁻⁴ while not directly stating that acacia is toxic *per se*, believes that it should not be used because of this toxicity, and the other,^{5, 6, 7} that this toxicity is due to faulty preparation or to the presence of some contaminant. The success of various clinics with the intravenous use of acacia lends support to the latter opinion. Meanwhile what the proper method of preparation should be and what contaminant causes this reaction is not known.

PROTOCOL.
Acacia-Glucose—Commercial.
Ampoule No. 1.

No. 22—16.5 kg (Happy), January 14, 1936.

Time	Temp. °F	W.B.C.	Remarks
10:30 A.M.	102.4	16	50 cc intravenously.
10:35			Vomitus, undigested food.
10:45			Depressed.
11:00			Vomitus, mucus.
11:15			Liquid stool, brown.
11:25	103.6	3.2	
11:30			Shivering, urine clear, small amount
12:00	104.8		
12:30 P.M.			Vomitus water, mucus.
1:00	106.4		Shivering
1:05			Light brown, unformed stool, slightly tinged with blood; vomitus bile, mucus; dog very depressed.
2:15	105.8		
3:25	104.3		

W.B.C. = Leucocytes in thousands.

¹ Lee, *J. A. M. A.*, 1922, **79**, 726.

² Hanzlik, De Eds, and Tainter, *Arch. Int. Med.*, 1925, **36**, 447.

³ Bernheim, *J. A. M. A.*, 1919, **73**, 172.

⁴ Studdiford, *Surg. Gynec. and Obstet.*, 1937, **64**, 772.

⁵ Bayliss, *J. Pharm. and Exp. Therap.*, 1920, **15**, 29.

⁶ Keith, Reports of the Special Investigation Committee on Surgical Shock and Allied Conditions, No. 1-8, Special Reprint Series No. 25. Wound Shock and Hemorrhage, Medical Research Committee, Oxford University Press, 285, 1919.

⁷ Huffman, *J. A. M. A.*, 1929, **93**, 1698.

The following study is an attempt to throw some light on the cause of this toxicity.

The symptomatology of a typical reaction in the dog is exemplified by the protocol. This experiment is one of the 4 reported by Studdiford⁴ in which a commercial preparation of gum-glucose which had caused 5 deaths and one marked reaction in the wards, was given to dogs. The amount given was 50 cc. The symptomatic picture is in marked contrast to Exp. 1 in the table in which 500 cc of non-reactive acacia was given.

The similarity of the train of symptoms in an acacia reaction to the "pyrogenic" reaction of reactive infusion fluids,⁸ reactive inulin,⁹ and the reaction following the intravenous administration of typhoid vaccine,¹⁰ leads to the question whether the acacia "reaction" might also be due to the pyrogen.

Accordingly, non-reactive acacia was inoculated with bacteria known to produce pyrogen, incubated for various periods of time,

TABLE I.
Bacterial Production of Pyrogen in Acacia.*

Organism	Incubation period	Wt of dog (kg)	Volume injected (cc)	Change in temp. (°F)	Changes in W.B.C. (× 1,000)	Symptoms
Control		12	500	100.6-100.0	9.7-9.3	None
<i>B. subtilis</i> (from culture slant)	96 hr	13	100	101.4-103.8	20.9-3.4	Marked shivering
Water organism "B" Genus unidentified (from culture slant)	96 hr	13	100	102.4-104.0	12.5-6.6	None
Water organism "C" Genus unidentified (from culture slant)	1 week at room temp.	12	90	101.0-105.0	25.5-5.7	"
<i>B. subtilis</i> and <i>Staph. albus</i> (contaminated by exposure)	48 hr	13.5	450	101.6-104.2	17.7-4.1	Marked shivering

* A sterile commercial 6% acacia in 0.85% NaCl solution was used throughout this series.

⁸ Co Tui, *et al.*, PROC. SOC. EXP. BIOL. AND MED., 1936, **35**, 297; *J. A. M. A.*, 1937, **109**, 250; *Ann. Surgery*, 1937, **106**, 1089.

⁹ Co Tui, *et al.*, PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 227.

¹⁰ Co Tui, *et al.*, *Ibid.*, in press.

filtered through a Berkefeld (W) candle to remove bacterial bodies, and then injected intravenously into the dog. The results in Table I show that the acacia after bacterial growth becomes "reactive".

Conclusions. 1. Acacia *per se* is not "reactive" or pyrogenic. 2. Commercial acacia prepared for intravenous use may be divided into 2 categories, reactive and non-reactive. 3. The growth in acacia of *B. subtilis* and 2 water organisms, all pyrogen-producers, changes non-reactive acacia into reactive acacia. 4. The febrile agent in reactive acacia is probably pyrogen.

10736 P

Formation of Peroxide and a Reversible Oxidation-Reduction in Solutions of Sulfanilamide.

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In accordance with a previous concept¹ of the mechanism of sulfhemoglobin formation I investigated whether sulfanilamide which often causes sulfhemoglobinemia forms H_2O_2 in presence of molecular oxygen. Aqueous solutions of sulfanilamide were prepared in different concentrations. The phenolphthalin reagent and, as a catalyst, copper sulfate, both as described by Schales,² were added. Oxygen was bubbled through the solutions. Parallel controls contained distilled water instead of sulfanilamide solution. The development of a red color resulting from the oxidation of phenolphthalin into phenolphthalein under the given conditions is specific for the presence, which implies the formation of hydrogen peroxide or labile peroxides. Concentrations of H_2O_2 as low as 1:10⁸ are detectable by means of this method.² In solutions containing sulfanilamide in a concentration of 250 mg per 100 cc and more the formation of H_2O_2 was regularly found. In lower concentrations, *e. g.*, 100 mg per 100 cc, no convincing specific effect was detectable.

From the failure to find H_2O_2 in the less concentrated solutions one could conclude that possibly not the sulfanilamide itself but rather

¹ Barkan, G., and Schales, O., *Hoppe-Seyler's Z. f. physiol. Chemie*, 1938, **254**, 241; **253**, 83; 1937, **248**, 96; Barkan, G., "Kongressbericht II" des XVI Internat. Physiol. Kongresses, Zürich (Schweiz), 1938, 250.

² Schales, O., *Berichte Dtsch. Chem. Gesellsch.*, 1938, **71**, 447.

some other substance accompanying the drug as an impurity might be responsible for the H_2O_2 formation as described above. Two facts make such a presumption improbable but do not entirely exclude it. (1) There could not be found any characteristic differences in the behavior of different preparations of sulfanilamide. (2) Neither could there be found any shift in the limit after purifying the drug by recrystallization.

Thus the formation of H_2O_2 , although under the given experimental conditions in a rather slight amount, seems to be due to the sulfanilamide itself. That makes it likely that *in vivo* by means of any catalyst present, H_2O_2 can be formed from an oxidation of sulfanilamide. An antecatalase activity of sulfanilamide or one of its oxidation products was claimed³ to be responsible for an accumulation of H_2O_2 formed by streptococci and pneumococci. Hydrogen peroxide formation quite independently of the presence of bacteria may explain the increase of the oxidation-reduction potential which was noticed not only in streptococcic cultures^{4, 5} but also in sterile broth⁵ in presence of sulfanilamide. H_2O_2 formation from the drug itself may take part also in the mechanism of both the chemotherapeutic and toxicologic actions of sulfanilamide.

Aside from the H_2O_2 formation there was found another rather striking phenomenon. In all solutions containing sulfanilamide, phenolphthalin reagent and copper, the reddish pink color of the alkaline phenolphthalein as developed by H_2O_2 changed more and more into violet-blue. At first within $\frac{1}{2}$ to 1 hour there is only a slight violet shade. The violet becomes more intense and after some hours, certainly on standing over night, usually a nearly pure blue color is formed. In no one of the tests containing sulfanilamide was the slow development of the violet-blue color in the previously red solutions missing. No corresponding change in the color of phenolphthalein was noticed if sulfanilamide was absent.

After cautiously adding small amounts of $\text{Na}_2\text{S}_2\text{O}_4$ the blue color disappears and a pure reddish-pink is formed again. Larger amounts of the reducing substance destroy the color throughout leaving a colorless or slightly yellow-brown solution.

³ Locke, A., Main, E. R., and Mellon, R. R., *Science*, 1938, **88**, 621; *Immunol.*, 1939, **36**, 183; Main, E. R., Shinn, L. E., and Mellon, R. R., *PROC. SOC. EXP. BIOL. AND MED.*, 1938, **39**, 272; Shinn, L. E., Main, E. R., and Mellon, R. R., *PROC. SOC. EXP. BIOL. AND MED.*, 1938, **39**, 591; 1939, **40**, 640.

⁴ Fox, C. L., Jr., German, B., and Janeway, C. A., *PROC. SOC. EXP. BIOL. AND MED.*, 1939, **40**, 184.

⁵ Warren, J., Street, J. A., and Stokinger, H. E., *PROC. SOC. EXP. BIOL. AND MED.*, 1939, **40**, 208.

By shaking with air, the reddish color is transformed within a few minutes into the previous violet-blue one. By adding again a small amount of $\text{Na}_2\text{S}_2\text{O}_4$ one can repeat the color change several times, always getting at first the red by reduction and then the blue by oxidation. After repeated transformations in both directions, however, mixed colors occur instead of the pure red and blue.

Using ascorbic acid as a reducing substance the same phenomena were observed; other reductants were less suitable. The reoxidation by shaking the reduced solutions with air seems to be a true autooxidation. However, one must consider that both $\text{Na}_2\text{S}_2\text{O}_4$ and ascorbic acid belong to those reducing substances which may react with molecular oxygen forming H_2O_2 .² It is not yet proved, but not excluded that the slow formation of the blue-colored substance on standing as mentioned above, may also be due to the influence of nascent H_2O_2 . There must be supposed some interaction of phenolphthalein, since sulfanilamide solutions even in presence of copper, under the same conditions do not change their color. On the other hand it is at least probable that the violet-blue colored substance is a similar if not the identical derivative as formed from sulfanilamide by ultraviolet irradiation in presence of oxygen.⁶

Even in the deep blue solutions the red phenolphthalein ions are still present. One can see spectroscopically the characteristic absorption band of phenolphthalein with the middle at about $554.7 \text{ m}\mu$. No other distinct absorption band is noticeable in the visible part of the spectrum, but a general absorption can be observed disappearing at once when after reduction the pure reddish color has come back.

On acidifying the reduced solutions after the red has reappeared the phenolphthalein as usual becomes colorless. By adding Na_2CO_3 solution cautiously while shaking slightly the blue color reappears almost instantaneously. The influence of pH upon the "auto-oxidation" and the mechanism of this process together with the peroxide formation is under further investigation.

⁶ Ottenberg, R., and Fox, C. L., Jr., *Proc. Soc. Exp. Biol. and Med.*, 1938, **38**, 479.

**Production of Experimental Poliomyelitis from
Untreated Stools.***

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It is now a well established fact that poliomyelitis virus may be present in considerable quantities in the stools of patients suffering from either paralytic or abortive attacks of the disease. These findings have been summarized by Trask, Vignec and Paul¹ who recently described a method for treating human stools in order to render them suitable for intraperitoneal inoculation into monkeys. Such procedures unfortunately involve the use of bactericidal substances of which the effect upon poliomyelitis virus is unknown. It is thus desirable to obtain a method of utilizing the material from stools without the intervention of measures which may cause attenuation or other change in virus. Such a method has been found in the simple intranasal inoculation of monkeys with an untreated stool suspension. On September 15, 1938, a stool specimen was obtained from a 3-year-old quadriplegic child through the courtesy of Dr. Harold Hobart of the Children's Hospital, Washington, D. C. The stool was collected on the third day after the onset of paralysis. At the time the child was still febrile. The specimen was immediately made up into a thick suspension with distilled water and the supernatant fluid was placed in the ice box. Two Rhesus monkeys were inoculated intranasally with this fluid on 3 successive days, each animal receiving a total of 3 cc per nostril. Following the introduction of the material the nasal passages were rubbed gently with a pipe cleaner.

On the fourteenth and eighteenth days respectively the animals developed typical but not extensive paralyses. They were killed and portions of the cord were removed for histological study and reinoculation. Typical poliomyelitic lesions were found in the grey matter of each spinal cord. Cultures of a suspension of each cord on blood agar and in blood broth were negative except for a *B. subtilis* contamination in one sample. Three mice were inoculated intracerebrally with 0.02 cc of 20% cord suspension from each

* Supported by a grant from the Commonwealth Fund.

¹ Trask, J. D., Vignec, A. J., and Paul, J. R., *J. Am. Med. Assn.*, 1938, **111**, 6.

case: all remained well although 2 monkeys inoculated intracerebrally and intraperitoneally with the same material developed typical poliomyelitis. In neither was the paralysis extensive although the cord lesions were characteristic.

It is thus possible to produce typical poliomyelitis in monkeys by the simple intranasal inoculation of untreated human stool. The method appears to be reasonably sensitive and should be applicable to the demonstration of small quantities of virus to which bactericidal measures might prove disastrous. It also makes it possible to work directly with poliomyelitis virus as it probably exists in transmission from one individual to another.

Although virus is present in human stools even in abortive cases,¹ various investigators² have been unable to demonstrate it in the feces of the macaque unless it was previously fed in large quantities. The direct intranasal inoculation of untreated monkey stool has likewise failed to reveal traces of virus in the intestinal contents at the height of paralysis. Three animals were inoculated intranasally on 4 successive days with 1 cc per nostril of an emulsion of stool taken from 2 animals which were prostrate after intracerebral introduction of MV virus. They showed no clinical signs of poliomyelitis although typical paralysis developed in controls receiving intracerebral inoculations of nerve tissue from the same cases.

The failure of virus to migrate from CNS to intestinal lumen in the macaque is consistent with the uniformly unsuccessful attempts² to infect this animal by the gastro-intestinal route. We have also been unable to produce poliomyelitis by means of MV virus enemas in 4 macaques which had previously been given a bloody diarrhoea by an 80% alcohol enema. These findings indicate quite conclusively that the Rhesus monkey is not readily susceptible to MV virus by way of the gastro-intestinal portal.

¹ Clark, P. F., Roberts, D. J., and Preston, W. S., Jr., *J. Prev. Med.*, 1932, **6**, 47; Leiner, C., and von Weisner, R., *Wiener klin. Wochens.*, 1910, **23**, 817; Levaditi, C., and Landsteiner, K., *Compt. Rend. Soc. de Biol.*, 1910, **68**, 311.

Neuronal Pathways as Determining Factors in Dissemination of Poliomyelitis in the Central Nervous System.*

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There has been hitherto no clear-cut evidence regarding the mode of progression of the virus of poliomyelitis from the portal of entry into and through the central nervous system (CNS) of man. In the experimental animal more information is available and there is now a general belief, supported by considerable evidence, that the principal rôle in the dissemination of the virus is played by neuronal pathways rather than by humoral ones, and that within the CNS there occurs a progression of the virus from the point of entry to certain susceptible regions, especially the motor centers in the hind-brain and spinal cord, where the most serious effects of the virus-host reaction become apparent. Little is known as yet concerning the determining factors in the transmission and localization of the virus throughout the CNS, although studies of some of the neuronal pathways involved have been made by Fairbrother and Hurst,¹ and by others. In this report, additional evidence bearing on these problems will be presented.

The material examined up to the present time consists of some 50 brains of Rhesus monkeys in preparalytic and paralytic stages of poliomyelitis, induced by introduction of the MV virus intranasally, intracerebrally, intraocularly and intraneurally. In a few cases the Wallingford strain (Trask and Paul²), inoculated intracerebrally and by skin rub, was used. The brains were prepared under optimal conditions for histological study chiefly by the gallo-cyanin method of Einarson,³ which satisfactorily demonstrates nerve cells, neuroglia, and inflammatory cells. In some cases various experimental procedures, such as section of the olfactory tracts, the corpus callosum, the bulbar pyramids, or the spinal cord, were carried out in order to modify if possible the mode of dissemination of the virus. In many cases microscopic sections of the olfactory bulbs, sympathetic ganglia, and other peripheral nervous struc-

* Supported by a grant from the Commonwealth Fund.

¹ Fairbrother, R. W., and Hurst, E. W., *J. Path. and Bact.*, 1930, **33**, 17.

² Trask, J. D., and Paul, J. R., *J. Bact.*, 1936, **31**, 527.

³ Einarson, L., *Am. J. Path.*, 1932, **8**, 295.

tures were available for study, in addition to the routine serial sections of brain and spinal cord. The analysis of the material has been made by study of the distribution of lesions and from the neuro-anatomical point of view, with the purpose of further elucidating the factors which govern the growth and dissemination of the virus in the CNS.

That the relation between the severity of the histopathological changes and the concentration of virus is not a strictly proportional one has been noted by Fairbrother and Hurst,¹ Schultz and Gebhardt,⁴ and others, and indeed is clearly apparent in our material. Nevertheless, there is usually a sufficiently close correspondence between the distribution of lesions in the CNS and the distribution of virus to permit a reasonably accurate analysis of the paths of dissemination of the latter, by means of histological studies. Fairbrother and Hurst in fact have regarded histological examination as superior in some instances to inoculation tests for detecting the presence of virus, especially in regions such as the cerebral cortex, which are highly resistant to the virus. Especially in the pre-paralytic stage, following the onset of fever, the absence of lesions in any particular center, although not strictly exclusive of the presence of virus, may be safely assumed to be correlated either with very low concentration or with the absence of virus.⁴

It is possible to mention only briefly some observations which serve to emphasize the value of careful study of typical lesions as evidence of virus passage along neuronal pathways in well-controlled material. (1) In monkeys in which poliomyelitis is induced by intranasal instillation, after section of one olfactory tract, there is a marked preponderance of lesions on the side of the intact tract in olfactory centers and in the hypothalamus as far back as the mid-brain tegmentum. This constant finding further supports previous evidence that the entry of virus into the CNS after intranasal instillation is by way of the olfactory tracts.⁵

(2) In pre-paralytic stages, after inoculation of virus into the leg region of area 4 (motor cortex), lesions are found in that portion of the dorsal thalamus, and that portion only, which has been shown

⁴ Schultz, E. W., and Gebhardt, L. P., *PROC. SOC. EXP. BIOL. AND MED.*, 1939, **40**, 577.

⁵ Schultz, E. W., and Gebhardt, L. P., *PROC. SOC. EXP. BIOL. AND MED.*, 1934, **31**, 728; Brodie, M., and Elvidge, A. R., *Science*, 1934, **79**, 235; Lennette, E. H., and Hudson, N. P., *PROC. SOC. EXP. BIOL. AND MED.*, 1935, **32**, 1444; Howe, H. A., and Eeke, R. S., *PROC. SOC. EXP. BIOL. AND MED.*, 1937, **37**, 125; Gordon, F. B., and Lennette, E. H., *J. Bact.*, 1938, **35**, 43.

by experimental anatomical studies to be directly connected with the site of inoculation (lateral part of nucleus ventralis posterolateralis of Walker⁶). The constancy and unexpectedly sharp localization of the lesions in these cases, along a well-established neuronal system, offer convincing evidence of the propagation of virus along neuronal pathways and of the validity, in certain instances at least, of the use of typical lesions as stigmata of even early virus invasion.

(3) Brodie⁷ has shown that after inoculation of virus into the motor cortex the concentration of virus at the height of paralysis is greater in the opposite motor cortex than in that of the side of inoculation. This agrees with our frequent finding in late paralytic stages that lesions are more numerous in the motor cortex opposite the side of inoculation. However, after experimental section of the corpus callosum, the great preponderance of lesions in the late paralytic stage is on the side of the inoculation. This indicates that the corpus callosum is the principal, but not exclusive, route for spread of the virus from one cerebral hemisphere to the other.

Since the predominant histopathological changes occur in the gray centers of the CNS rather than in the fiber pathways which interconnect them, it is necessary always to keep in mind the patterns of interconnections of the nerve cells, in order to be able to interpret the paths of progression of the virus from center to center, if it be assumed that the virus is transmitted along neuronal pathways. This has been rendered highly probable by evidence that nerve cells rather than any other components of nervous tissues are necessary as a substrate for virus growth.⁸ In our material this fact is most clearly manifested by an interesting and previously unreported finding,—namely, that there is a complete absence of any signs of virus invasion in those centers of the optic thalamus in which all of the nerve cells have undergone previous retrograde degeneration and have disappeared following destruction of their axonic terminals in the cerebral cortex. Many instances of this phenomenon have been observed, even in those thalamic centers which always contain lesions in paralytic stages following intracerebral inoculation.

The lesions produced by the virus of poliomyelitis in the CNS of the Rhesus monkey are found principally along 2 preferential systems of neuronal pathways. This is best demonstrated as a rule

⁶ Walker, A. E., *The Primate Thalamus*, 1938, University of Chicago Press, Chicago.

⁷ Brodie, M., *J. Immunol.*, 1933, **25**, 71.

⁸ Hurst, E. W., *J. Path. and Bact.*, 1929, **32**, 457.

only in preparalytic and in early paralytic stages, in which the sharpness of localization of lesions along these pathways is especially evident. Later, the elementary pattern of progression of the virus may be obscured by its spread into secondary or tertiary pathways leading from the principal ones. The pre-paralytic intranasally inoculated animals, following the onset of fever, show the most important preferential pathway of virus passage in the CNS. This is the pathway from olfactory bulb to the spinal cord by way of the anterior perforate substance (tuberculum olfactorium), the pre-optic area, the hypothalamus and the descending pathways from these centers. These appear to be the olfacto-tegmental and hypothalamo-tegmental tracts, and the periventricular bundle of Schütz, which connect with the midbrain tegmentum and the reticular formation of the hindbrain. From the latter regions virus passes to the motor centers of the medulla and spinal cord, apparently by way of many short connections.

After intranasal instillation of virus, in the late preparalytic stage, lesions not on the primary pathways are found chiefly in the parolfactory regions and in the amygdaloid nuclei, due to spread of virus from the olfactory bulb and the tuberculum olfactorium, and also in the midline gray of the dorsal thalamus. The virus appears to reach the latter by passage along the periventricular fiber system, which connects the hypothalamus with the midline centers of the thalamus. There is rarely seen any spread of lesions from the midline thalamic centers to the more laterally placed sensory portions of the thalamus, and then only in late paralytic stages. The anterior thalamic nuclei are occasionally the site of inflammatory infiltrations, apparently due to spread of virus from the midline thalamic centers, since the mammillary bodies which also connect with the anterior thalamic nuclei are, with rare exceptions, conspicuously free of lesions. There is no evidence in our material that the principal path of virus spread to the spinal cord in these intranasal cases is the spinothalamic tract, as suggested by Faber,⁹ since lesions are not found in the somatic sensory (ventrolateral) portions of the optic thalamus until paralysis is extreme, and then only when lesions are also present in the cortex around the central sulcus. Occasionally in cases of extreme paralysis, lesions in the thalamus may be present only in the midline centers, which have no direct connections with lower levels of the CNS. It is thus apparent that subinoculation tests made with material from unspecified portions of the thalamus

⁹ Faber, H. K., *Medicine*, 1933, **12**, 83.

can not be critical in the analysis of virus propagation to or from the thalamus.

The second important preferential pathway is that from the motor cortex to the medulla and spinal cord. Along this pathway lesions are found not only after intracerebral inoculation into any part of the cortex, but also, in late paralytic stages, after inoculation by any other portal, including the intranasal. In fact, unlike other parts of the cerebral cortex, the motor cortex (areas 4 and 6) appears to be a favorable site for virus growth. In prodromal stages after intracerebral inoculation, the distribution of lesions suggests that the pathway from the motor cortex to lower centers is by way of the globus pallidus, the zona incerta, the field of Forel, the substantia nigra, and the midbrain tegmentum, rather than by way of the more direct cortical-spinal route. Although the latter is not excluded as a possible route of dissemination of the virus, it is by no means necessary for virus spread from motor cortex to the opposite side of the spinal cord, since the pattern of crossed initial paralysis does not appear to be materially affected by section of the appropriate bulbar pyramid.¹⁰ The chief pathways of virus spread from the subthalamic and midbrain regions, as evidenced by the distribution of lesions, are apparently the same as those involved after intranasal instillation.

In summary, the sequence of events appears to be as follows: In the preparalytic stage, after the onset of fever, the pattern of propagation resulting from inoculation by different portals may be recognizably different, with sharp localization of lesions along certain neuronal pathways. By the time paralysis has set in, regardless of the portal of entry, the virus has reached the preferential pathways from the olfactory centers to the spinal cord, and from the motor cortex to the spinal cord, and has passed both rostrally and caudally along these pathways to the centers involved in a typical intranasal or intracortical inoculation. In late paralytic stages the primary pathways of transmission of the virus are obscured by the fact that evidences of virus invasion are found in centers other than those along the preferential systems of nerve cells and fibers. This indicates a spread, after the initial invasion, from the more susceptible primary pathways, to less susceptible centers. Finally, unless recovery occurs, an overwhelming spread of the virus occurs from the centers primarily and secondarily involved, so that even some cen-

¹⁰ Howe, H. A., and Ecke, R. S., *PROC. SOC. EXP. BIOL. AND MED.*, 1937, **37**, 123; also Howe, H. A., and Ecke, R. S., unpublished experiments.

ters which are rarely or never affected in the early stages (that is, centers with high immunity) are reached by the virus and succumb to it. Thus, the final pathological picture in all cases is similar, as apparently the susceptibility to virus of various parts of the CNS is independent of the portal of entry (see also Pette, Demme, and Környey¹¹). However, even terminally it is possible to observe in certain cases differences in the distribution of lesions, depending on the portal of entry. For example, if the virus is inoculated by some other portal than the intranasal one, signs of virus invasion in the late paralytic stages may occasionally be observed as far forward in the olfactory system as the tuberculum olfactorium, but, in contrast with intranasal cases, apparently never in the olfactory bulbs.¹²

10739 P

The Pia-Arachnoid as a Barrier in Experimental Poliomyelitis.*

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The occasional demonstration of poliomyelitis virus in the cerebrospinal fluid of experimental animals has given rise to a series of experiments and speculations regarding the rôle of this body liquid in the dissemination of the disease through the central nervous system. Such investigations as those of Clark and Amoss¹ and Hurst² who produced experimental poliomyelitis regularly by intracisternal and intrathecal inoculation have led to the assumption recently made articulate by Schaeffer and Muckenfuss³ that intracerebral inocula may be effective at sites far distant from the point of introduction. In previous observations the permeability of the ependyma and the possibility of injury to the pia-arachnoid are factors which have not been properly controlled. The following experiments indicate that under ordinary conditions the leptomeninges

¹¹ Pette, H., Demme, H., and Környey, St., *Deutsche Z. f. Nervenhe.*, 1932, **128**, 125-252,

¹² Sabin, A. B., and Olitsky, P. K., *J. Am. Med. Assn.*, 1937, **108**, 21.

* Supported by a grant from the Commonwealth Fund.

¹ Clark, P. F., and Amoss, H. L., *J. Exp. Med.*, 1914, **19**, 217.

² Hurst, E. W., *J. Path. and Bact.*, 1932, **35**, 41.

³ Schaeffer, M., and Muckenfuss, R. S., *Am. J. Path.*, 1938, **14**, 227.

constitute an effective barrier—so effective that large amounts of active virus may be present in the cerebrospinal fluid without any clinical indication of poliomyelitis.

These experiments fall into 3 groups: (a) virus dripped over an exposed cortical surface with intact pia; (b) virus introduced into the lumbar cistern with intact pia; (c) virus introduced at either of the above sites after deliberate pial injury.

Fifteen animals were used in the first group. The cerebral cortex was exposed by opening an oval dural flap 3 cm by 2 cm which was centered over the paracentral lobule with the long axis in a rostro-caudal direction. Great care was taken to avoid injury to the pia, although the arachnoid was undoubtedly torn in places. One-quarter to three-quarters cc of 20% MV virus in salt solution was then dripped over the exposed cortical surface, the edges of the dural flap being elevated to insure as wide dissemination as possible. The dura was then carefully sutured and the incision closed. Eight controls were prepared by the direct intracerebral inoculation of 0.25 cc and 2 by intranasal inoculation of the same virus suspensions. All but 2 of the controls became paralysed within 4-9 days while the operated animals remained well over a period varying from 11 days to 4 months. In 9 instances fever was recorded but there was no suggestion of paralysis.

The second group comprised 7 animals in which virus was introduced into direct pial contact in the lumbar subarachnoid space. Three of these animals received 0.5 cc of active virus dripped over the exposed lumbar enlargement. This type of procedure had the disadvantage that most of the virus escaped into the surrounding muscles although an infective dose undoubtedly came in contact with the cord. In this group 2 direct cord inoculations resulted in paralysis while the animals receiving virus drips remained free of either fever or paralysis. In order to introduce larger quantities of virus intrathecally, 4 animals were prepared with an exposure of the dura of the lumbar cistern. This was then carefully nicked and cerebrospinal fluid was allowed to escape freely. A blunt curved needle was then inserted through this small opening and 0.5 cc of active 20% MV virus in salt solution was dispersed among the roots of the cauda equina. In this way complete retention of the inoculum was obtained. A control was prepared by direct piqure of 0.4 cc of the same virus into the sacral cord. As in the previous series, the control succumbed within 4 days and the experimental animals showed neither fever nor paralysis.

Of the 22 animals which resisted the introduction of virus into

the cerebrospinal fluid 11 were sacrificed for histological study: 11 were subsequently given intracerebral or intranasal inoculations. Two of these were resistant to infection while the remaining 9 contracted typical poliomyelitis.

The last group is concerned with animals in which pial injury was produced either accidentally or deliberately. In the cortical drip experiments the dura was not sutured in 5 cases. Although the inoculum was reduced to 0.05 cc of 20% virus in 3 of these animals, paralysis resulted in each instance. At autopsy the chief finding was a cortical herniation which was either very hyperaemic or frankly necrotic. In 2 instances a suture needle injury to the cortex was followed by paralysis.

The idea that infection was made possible by a change in pial permeability is confirmed by the following experiment. In 3 animals 0.5 cc of a distilled water suspension of 20% MV virus was carefully introduced into the lumbar cistern by the technique already described. In each instance paralysis resulted although autopsy revealed no visible damage to the cord or nerve roots.

The protective barrier of the pia is thus conceived to be largely a mechanical one, and under the influence of factors which bring about changes in its permeability. Under normal conditions it is probably effective enough to render the cerebrospinal fluid of the subarachnoid space negligible as a virus-disseminating medium. These findings may be interpreted as contributing further evidence for the neurotropism of poliomyelitis virus. The resistance of the ventricular ependyma will be similarly considered in succeeding experiments.

10740

Penetration of Sesame Oil Painted on the Capon Comb

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(Introduced by N. A. Michels.)

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Androgenic substances, extracted from human urine by a method previously reported,¹ were dissolved in sesame oil and applied to the surface of the combs of white leghorn capons. Since percutaneous

¹ McCahey, J. F., Hansen, L. P., and Soloway, D., *J. Urol.*, 1937, **38**, 397.

application of androgens, dispersed in oil or other fatty vehicle, has been used both for assay on the combs of chicks and capons, and as a method of therapy in man, it is not only of interest to know how effective the androgens are when so applied, but also what happens to the vehicle. The time required to induce growth and the actual increase in size of the comb, are measures of the rapidity and adequacy of penetration of the androgens through the various layers of the skin, while histological studies with special stains for fatty substances indicate the fate of the sesame oil.

The greater growth of the capon comb in response to surface applications of androgens, as compared with the growth obtained from parenteral administration has been noted.^{2, 3, 4, 5} Table I shows the growth of a capon comb resulting from anointment with urinary androgens, compared with the response obtained previously in the same bird when 4 times the amount of the same material was injected. The total length of the comb and the height of each of its points were measured in millimeters for all birds used for several weeks before the applications and daily thereafter until the animals were sacrificed. Note that the response was from 2 to 3 times greater with anointment than it was with injections.

TABLE I.
(Capon No. 5.)
Comparison Between Growth Response of Capon Comb to Intramuscular Injections and Direct Application of Urinary Androgens.

Date Injection	11/1	11/11 1 cc	11/12 1 cc	11/14	11/15	11/16	Max. growth, mm
Total length, mm	57.5	58.0	61.0	67.0	67.0	67.0	9.0
Height of points, mm							
No. 1	26.5	28.0	29.0	35.5	35.0	34.5	7.5
2	26.5	27.5	28.0	33.0	32.5	33.5	6.0
3	25.8	27.0	27.0	32.3	32.5	33.0	6.0
4	22.3	22.5	24.0	28.8	28.0	29.0	6.5
Date Application	1/31	2/17 ¼ cc	2/18 ¼ cc	2/20	2/21	2/24	Max. growth, mm
Total length, mm	64.5	64.5	69.0	79.0	82.0	82.0	17.5
Height of points, mm							
No. 1	31.5	32.0	36.0	45.5	46.5	47.0	15.5
2	29.0	30.0	34.5	42.5	43.0	46.0	16.0
3	29.0	29.0	34.5	42.0	43.0	44.5	15.5
4	25.0	25.0	31.0	39.0	41.0	42.0	17.0

² Fussgänger, R., *Medicine in Its Chemical Aspects*, I. G. Farbenindustrie A. G., 1933, 1, 198; 1934, 2, 185.

³ Soloway, D., Hansen, L. P., and McCahey, J. F., *Anat. Rec.*, 1936, **64**, 46.

⁴ Dessau, F., and Freud, J., *Acta brev. Neerland. Physiol.*, 1936, **6**, 9.

⁵ Deanesley, R., and Parkes, A. S., *Proc. Roy. Soc., B*, 1937, **124**, 279.

The combs of 12 capons (which showed complete absence of testes at autopsy) were painted with $\frac{1}{4}$ cc of the sesame oil-containing extract representing the androgens recovered from 125 cc of male student urine. Small drops of the material, from a delicate syringe, were placed upon the surface of the comb and spread with a very fine camel's hair brush over the whole surface, avoiding pressure or rubbing. Thus a thin film of oil was placed upon the most superficial portion of the epidermis of the comb. One comb was removed 3 hours after the application, one at 6, one at 9, one at 12 hours after. The rest of the combs received another similar application 24 hours later after which one comb was removed at the end of each successive 24 hour period. Thus, various stages showing penetration of the oil as well as reaction within the comb to androgens were obtained within the limits of 3 to 188 hours.

Several combs were painted with sesame oil only, and numerous untreated, unstimulated capon combs were also sectioned.

Frozen sections of a piece of each comb were stained with Sudan III, Scharlach R and Cyanin. Other pieces were placed in various fixatives (formalin, Zenker's-formol, Bouin's, Hermann's, Champy's, etc.) and in aqueous solutions of Osmic acid. Sections from each piece were stained with hematoxylin and eosin, Mallory's, iron hematoxylin and mucin stains.

Results. Although definite growth of the comb, wattles and earlobes was evident within 24 hours after the first application, demonstrating the rapid penetration and stimulating activity of the hormones, the sections of the comb stained for the sesame oil showed no penetration beyond the stratum corneum. The stained oil stopped abruptly at the inner limit of this layer. The entire area of the stratum corneum was orange to scarlet with Sudan, blue with Cyanin, and varying shades of dark brown to black with Osmic acid (the color depending upon the amount of oil applied and the thickness of the sections). The penetration of the oil through this keratinized layer occurs within the first 3 hours following the initial application. No clear evidence of any oil in or between the rest of the epidermal cells or in the dermis could be demonstrated at any of the stages studied.

Much of the oil seemed to accumulate and persist in the spaces between the projecting surface papillae, and here, keratin-like granules within the most superficial cells adjoining the inner margin of the cornified layer occasionally showed a slight affinity for the Sudan stain. The white, thickened (extra-keratinized) layer, char-

acteristic of the capon comb, was rapidly made translucent by the oil so that the underlying epithelial cells and the capillaries in the dermis became fairly visible and the shrunken, closely approximated surface papillae more evident.

The same amount of penetration of the sesame oil, when used alone, was noted. The stained sections of untreated, unstimulated, unoiled combs suggest the presence of some substance in the stratum corneum that reacts slightly with Osmic acid, giving a brown color, but not the black of combs painted with the oily solution. This reaction may be due to cholesterol or keratin present in the horny layer.

Although the sesame oil could not be demonstrated, by the methods used, in or between the deeper cells of the epidermis or in the dermis, this does not remove the possibility that absorption of the oil may occur in an unstainable form. However, there is a marked color reaction still present in the cornified layer 188 hours after the first application, indicating the presence of most of the oil. Studies^{6,7} on the absorption through the human skin indicate that the type of oil represented by sesame does not penetrate the barrier of the skin. Besides, there are no gland openings or hair follicles to aid absorption in the comb, and although there are some feathers near the base of the comb, care was exercised to avoid painting them.

Removal of sesame and similar oils injected into connective tissue and muscle, or used as vehicles for hormones, has been investigated^{8, 9, 10, 11, 12, 13} with the conclusion that they undergo a very slow absorption and penetration even within these internal structures. Similarly, studies of absorption of such oils from mucous membranes also indicate comparable behavior.^{14, 15}

The urinary androgens are probably slowly partitioned from the oil at the inner border of the stratum corneum and then selectively absorbed through the rest of the epithelial cells into the dermis and its capillaries. The ability of crude urinary extracts of androgens to penetrate the epidermis and spread through the dermis may rep-

⁶ Sollmann, T., *A Manual of Pharmacology*, 1927, 3rd ed., p. 76.

⁷ Macht, D. I., *J. A. M. A.*, 1938, **110**, 409.

⁸ Korenchevsky, V., Dennison, M., and Schalit, R., *Biochem. J.*, 1932, **26**, 1306.

⁹ Korenchevsky, V., Dennison, M., and Kohn-Speyer, A., *Biochem. J.*, 1933, **27**, 778.

¹⁰ Deanesly, R., and Parkes, A. S., *J. Physiol.*, 1933, **78**, 155.

¹¹ Bülbring, E., and Burn, J. H., *J. Physiol.*, 1935, **85**, 320.

¹² Deanesly, R., and Parkes, A. S., *Lancet*, 1936, 230, **1**, 837.

¹³ Parkes, A. S., *Brit. Med. J.*, 1938, **1**, 371.

¹⁴ Eleftheriou, D. S., *Compt. rend. Soc. Biol.*, 1936, **123**, 231.

¹⁵ Eleftheriou, D. S., *Compt. rend. Soc. Biol.*, 1936, **123**, 1186.

resent a property similar to the spreading effect (R-factor) first obtained by Duran-Reynals¹⁶ and others from testis tissue extracts injected intradermally. McClean¹⁷ described the histology of an area of the dermis of the rabbit similarly injected, wherein the collagenous bundles were thought to separate into smaller divisions and fibrillae, resembling very early changes noted in the tissues of the dermis of the capon comb under influence of androgens.

Conclusions. (1) Sesame oil is not absorbed (at least within 188 hours) beyond the stratum corneum of the epidermis of the capon comb, following surface application of the oil alone or the oil acting as a vehicle for androgens. (2) Urinary androgens are selectively absorbed from sesame oil at the inner margin of the stratum corneum, then rapidly penetrate to induce characteristic growth changes. (3) The ability of crude urinary extracts used in these experiments to penetrate the barrier of the epidermis and spread in the dermal and subdermal tissues is similar to the spreading effects of intradermally injected testicular extracts. (4) The greater growth reaction in the comb due to percutaneous applications as compared with parenteral administration of the same androgenic material, appears to be a matter of rate of absorption more nearly approaching the normal physiological requirements of the bird. This involves a gradual and adequate separation of the hormone from the oil, a rapid penetration of the rest of the epidermis and dermis and a large dermal network of capillaries, present even in the capon crest.

The authors wish to thank Professor J. Parsons Schaeffer for his interest and aid in this work and Dr. John Franklin Huber for helpful criticism.

¹⁶ Duran-Reynals, F., *J. Exp. Med.*, 1929, **50**, 327.

¹⁷ McClean, D., *J. Path. and Bact.*, 1931, **34**, 459.

The Progesterone-Like Activity of Desoxycorticosterone.*

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The structure of many chemicals obtained from the adrenal cortex has been determined. The similarity of certain of these substances to progesterone has been noted. One of the more interesting chemicals, desoxycorticosterone was recently obtained from the adrenal¹ after having been previously prepared synthetically.

The experiments reported here demonstrate the progestational activity of desoxycorticosterone and the oestrous receptivity response^{2, 3} in the guinea pig which has previously been considered specific for progesterone.⁴

Immature female rabbits and young adult spayed guinea pigs were used. The rabbits were given daily injections of 8.33 or 25 gamma of estradiol benzoate‡ for six days and the various amounts of desoxycorticosterone§ over a period of 6 days (Table I). Two rabbits received the estradiol benzoate alone and two received 1 mg and 0.5 mg progesterone following the estrogen. At biopsy sections of the uterine horns were removed, sectioned and rated as to the extent of progestational proliferation as previously described.^{5, 6}

Six to 10 mg of desoxycorticosterone gave a progestational response approximately equivalent to 1 mg of progesterone (+++ to +++) and the uteri of the rabbits receiving 3 mg of desoxycorticosterone resembled those of animals receiving 0.5 mg proges-

* This investigation has been supported by the Belgian American Educational Foundation, the Anna Fuller Fund, and the Jane Coffin Childs Memorial Fund.

† Belgian American Educational Foundation Graduate Fellow.

¹ Reichstein, T., and v. Euw, J., *Helv. Chim. Act.*, 1938, **21**, 1197.

² Dempsey, E. W., Hertz, R., and Young, W. C., *Am. J. Physiol.*, 1936, **116**, 201.

³ Collins, V. J., Boling, J. L., Dempsey, E. W., and Young, W. C., *Endocrinology*, 1938, **23**, 188.

⁴ Hertz, R., Myer, R. K., and Spielman, M. A., *Endocrinology*, 1937, **21**, 533.

‡ The estradiol benzoate (progynon B) and progesterone (Proluton) were generously supplied by Drs. E. Schwenk and M. Gilbert of the Schering Corporation.

§ The desoxycorticosterone was furnished through the courtesy of Dr. E. Oppenheimer by the Ciba Pharmaceutical Products, Inc.

⁵ Allen, W. M., *Am. J. Physiol.*, 1930, **92**, 612.

⁶ McPhail, M. K., *J. Physiol.*, 1934, **88**, 145.

PROGESTERONE-LIKE ACTIVITY OF DESOXYCORTICOSTERONE 553

TABLE I.
Uterine Responses of Immature Female Rabbits Receiving 6 Daily Doses of
Estrogen Followed by 6 Daily Doses of Desoxycorticosterone.

No. of tests	Daily amt estrogen (gamma)	Total dosage of desoxycorticosterone (mg)	Proliferation rating
1	8.3	30	++
2	8.3	10	++ and +++
1	25.0	10	+++
1	8.3	6	++
1	25.0	6	++++
2	8.3	3	+ to ++
2	25.0	3	+ to ++
1	8.3	0.6	0

terone (+ to ++). Other studies have indicated the progesterone-like activity of desoxycorticosterone.^{7, 8}

In studying the effect of desoxycorticosterone on the estrous response of guinea pigs the animals were, as a preliminary procedure, conditioned by injections of 50 I.U. of estrone and 24 hours later were injected with 0.5-2.0 mg of desoxycorticosterone dissolved in corn oil. Oestrous responses were elicited in at least one animal from each dose group thus showing that the substance had definite progesterone-like activity. Ten animals were then injected with 1 mg of desoxycorticosterone 24 hours after a conditioning dose of 50 I.U. of estrone and 60% of the animals so treated gave the heat response. When this percentage is referred to the dose-response curve of progesterone it is found that 1 mg of desoxycorticosterone is approximately equivalent to 0.1 mg of progesterone³ and it may be concluded that this cortical hormone as measured by this reaction has at least one-tenth the potency of progesterone (Table II).

TABLE II.
Effect of 1.0 mg of Desoxycorticosterone Dissolved in ½ cc of Corn Oil in Causing Heat in Spayed Guinea Pigs When Injected 24 Hours After a Conditioning Dose of 50 I.U. of Theelin.

No. of animals injected	No. in heat	Mean latent period	Mean length of heat	Combined latent and length
10	6	7.3 hr	3.6 hr	10.9 hr

It is to be noted that the latent period or interval between injection of desoxycorticosterone and the elicitation of the heat response was 7.3 hours which is somewhat long when compared with 4.5 hours for progesterone; also the length of heat, 3.6 hours, is short when compared with 6.59 hours for progesterone. However, the

7 Miescher, K., Fischer, W. H., and Tschopp, E., *Nature*, 1938, **142**, 435.

8 Wells, J. A., and Greene, R. R., *Proc. Am. Physiol. Soc.*, 1939, 236.

combined latent period and length of heat for the 2 substances agrees quite closely: for progesterone it is 11.09 hours; for desoxycorticosterone it is 10.90 hours.

Summary. Desoxycorticosterone resembles progesterone in that it produces progestational proliferation in the endometrium of immature rabbits and induction of the oestrous receptivity response in spayed guinea pigs. The above experiments indicate that desoxycorticosterone is from 1/6 to 1/10 as potent as progesterone.

10742 P

A New and Effective Method of Treating Canary-Pox.

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Canary-pox is a variety of fowl-pox, and is capable of causing very severe loss to those who raise canaries or other small birds. Fowl-pox inflicts considerable losses on poultrymen each year, both because of the deaths it causes and the reduction in egg-laying by affected birds. Up to the present there has been no effective treatment for any variety of the disease, although temporary immunization of chickens is possible.

In canaries the pox is highly fatal, the mortality being almost 100%, and also highly contagious. The disease occurs in 2 or possibly 3 forms. In one the earliest indication is a small swelling of the marginal epithelium about the eyes. This rapidly increases in size until within 3 or 4 days the eye is completely closed, and then continues to spread until death occurs which is usually within a week or 10 days. Or a similar nodule may appear about the nostrils, or at the angles of the mouth; and run a rather similar course.

In other canaries the first indication of the disease appears when the bird begins to gasp, and here the fatal outcome of the infection is often even more prompt. Occasionally birds are also seen with scaly or warty growths about the toes and legs, but although this is said to be a manifestation of the same disease, and is frequently associated with epidemics in which cases of the two first-mentioned types are numerous, it runs a much slower course. But here, too, the bird eventually dies, though not perhaps for some weeks or months. In the meantime the claws and even the toes are frequently lost, and the

* Aided in part by a grant-in-aid from the American Philosophical Society.

bird becomes unable to perch. Apparently few studies of canary-pox have so far been made, the only ones known to the authors being those of Kikuth and Gollub,¹ Burnet,² and Reis and Nobrega.³ Correspondence with Dr. Beaudette of the New Jersey Experiment Station and others, however, indicates that the disease is probably of rather frequent occurrence in aviaries, and there is reason to believe that it is also common among wild birds.

Diagnosis of the disease was made not only from the character of the lesions, but also from inclusion bodies. These are of 2 sorts in fowl-pox, larger bodies known as Bollinger bodies, and smaller ones within these which have received the name of Borrel bodies. Filterability of the infectious agent has not yet been established by us, but other authors (Irons⁴) have found this difficult. The strain of the virus used in these experiments proved quite specific when inoculated into chickens, only a very transitory lesion being produced. Canaries could be infected by injection of suspensions of tissue from lesions of other canaries, by injection of blood, or by direct contact of a lesion with the eye of a clean bird. It is probable that infection occurs naturally by such contact, or by contact with perches, or other parts of the cage, since infected birds are always rubbing their heads and bills on such objects. Or infection may take place from contaminated food and water, and quite possibly from mites.

Treatment with mercurochrome has proved highly effective. Of 26 cases in which it has been tried only 3 have died, and these were far advanced when it was started. Alcoholic solutions of 1½% and 3% mercurochrome have been used, and also Scott's solution, but the alcoholic solutions seem more suitable for application. Usually a swab is used, or the solution may be applied between the cornea and the conjunctiva with a small blunt dropper. The amount of treatment needed depends on the case. A week is often enough, but 3 or even more may be required if the case is well advanced when it is first started. Two applications each day are usually sufficient. Cases of both the ophthalmic and the gasping type have been treated with success, and the results are often dramatic. Since the lesions are in the pharynx in the second type of the disease we have allowed birds so affected to swallow a little mercurochrome. Our experience thus far indicates that for this disease this drug comes close to being an almost perfect specific.

¹ Kikuth, W., and Gollub, H., *Zentralbl. f. Bakt.*, I Orig., 1932, **125**, 313.

² Burnet, F. M., *J. Path. and Bact.*, 1933, **37**, 107.

³ Reis, J., and Nobrega, P., *Arch. Inst. Biol.*, 1937, **8**, 211.

⁴ Irons, Vernal, *Am. J. Hyg.*, 1934, **20**, 329.

Effect of Testosterone Propionate on Fallopian Tube Peristalsis.

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In a previous study it has been shown that estrogenic hormone initiates and controls peristalsis of the fallopian tubes. This was demonstrated by producing normal tubal contractions in women after the menopause following the administration of estradiol benzoate.¹ At present we wish to report on the effect of testosterone propionate on normal tubal peristalsis.

It has been shown that testosterone propionate, when administered in adequate amounts to women with normal menstrual cycles, suppresses menstruation,^{2, 3, 4} induces regressive changes in the endometrium² and estrogen deficiency changes in the vaginal smear.^{4, 5} We were interested to find out if testosterone propionate would inhibit the muscular contractions of the Fallopian tubes.

A series of 5 women who were menstruating regularly were selected for this study. A preliminary tubal contraction record was obtained between the 7th and 10th day of the cycle. Tubal contractions were recorded on a kymograph, using the Rubin insufflation (carbon dioxide) technique.⁶ Thereafter the patients were given intramuscular injections of testosterone in oil,* in doses of 50 mg 3 times weekly. Tubal contraction recordings were taken at intervals of 7 to 14 days.

In all of the cases tubal contractions were completely inhibited when the patients had received approximately 500 mg of testosterone propionate. With smaller doses (approximately 200-300 mg) the contractions were reduced in amplitude and frequency.

It appears from these observations that testosterone propionate counteracts the muscle-stimulating action of estrogenic hormone, resulting in a suppression of the normal tubal contractions.

¹ Geist, S. H., Salmon, U. J., and Mintz, M., *Am. J. Obs. and Gyn.*, 1938, **36**, 67.

² Gaines, J. A., Salmon, U. J., and Geist, S. H., *PROC. SOC. EXP. BIOL. AND MED.*, 1938, **38**, 779.

³ Loeser, A. A., *Lancet*, 1939, **1**, 373.

⁴ Papanicolaou, G. N., Ripley, H. S., and Shorr, E., *PROC. SOC. EXP. BIOL. AND MED.*, 1938, **37**, 689.

⁵ Salmon, U. J., Walter, R. I., and Geist, S. H., *PROC. SOC. EXP. BIOL. AND MED.*, 1938, **39**, 467.

⁶ Rubin, I. C., *J. A. M. A.*, 1929, **92**, 1597

* For the testosterone propionate used in this investigation, we are indebted to Dr. Erwin Schwenk, Schering Corporation, Bloomfield, N. J., and Mr. Robert C. Mautner, Ciba Pharmaceutical Products, Summit, N. J.

10744 P

Experimental Studies on Bronchomonoliasis.

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Infection of the respiratory tract by pathogenic monilia gives rise to the disease entity designated bronchomonoliasis. The first report on record of this disease was given by Castellani¹ from Ceylon. In this country it was primarily reported by Boggs and Pincoffs.² Flinn³ reported 9 cases. Monoliasis is no longer considered endemic in the tropics since it is now being reported with increasing frequency from all parts of the world.

With a strain of *Monilia albicans* isolated from a proven case of bronchomycosis we injected rabbits intravenously, intraabdominally, intratracheally and directly into the lung parenchyma with varying doses of the recovered culture. The routes, other than intravenous, proved either inconsistent in their effects or the animals remained apparently normal. As the intravenous method of inoculation yielded constant results, this route was employed as the method of choice. The dosage was measured according to the method of Stovall.⁴

No lesions have been described which are specific or pathognomonic of bronchomonoliasis. It is, however, generally agreed that the gross appearance of the lesions resembles tuberculosis. Mendelson⁵ pointed out that the lesions are, in reality, mycotic tumors and as a rule show no signs of breaking down. Ikeda⁶ found miliary cortical abscesses of the kidney to be the most conspicuous finding in animals injected by various routes.

In the present work the injected rabbits were divided into 2 series.

Series I: These animals were given the lethal dose of *Monilia albicans* intravenously as established by Stovall,⁴ and died within 24

¹ Castellani, A., *Fungi and Fungous Diseases*, Chicago, American Medical Association, 1927, p. 121.

² Boggs, T. A., and Pincoffs, M. C., *Bull. Johns Hopkins Hosp.*, 1915, **26**, 407.

³ Flinn, John W., Flinn, Robert S., and Flinn, Z. Mackay, *Ann. Int. Med.*, 1935, **9**, 42.

⁴ Stovall, W. D., and Pessin, S. B., *Am. J. Clin. Path.*, 1933, **3**, 347.

⁵ Mendelson, R. W., *J. A. M. A.*, 1921, **77**, 110.

⁶ Ikeda, Kano, *Arch. Path.*, 1936, **22**, 63.

to 48 hours. The lesions found at autopsy were usually in the periphery of the liver, kidney or lung. While the gross appearance of the small nodules resembled miliary tubercle formation, the histopathology, however, consisted of central necrosis with polymorphonuclear neutrophilic and slight lymphoid cellular reaction at the periphery.

The tendency to peripheral location in the organs was striking. In the instance of the kidneys an embolic formation of mycotic microorganisms might form an explanation; this interpretation would not apply, however, to the blood circulatory arrangement of the liver or lung.

Series II: These rabbits were injected with only approximately 5% of the dose administered in series I. Death did not take place for 10 days to 3 weeks. In these animals we found the typical mycotic tumors, resembling miliary tubercles, in all the parenchymatous organs. Microscopically the lesions showed peripheral lymphocytic infiltration, epithelioid proliferation and giant-cell formation, thus simulating the histopathology of a miliary tubercle.

In the lesions of both series of animals monilia occasionally can be noted with hematoxylin-eosin staining but it is usually necessary to resort to the Gram-Weigert method to demonstrate the microorganisms.

While the gross lesions present in both series of animals revealed some similarity to miliary tubercles, in that they were rounded, white and firm, the histopathologic aspect varied according to the period of survival of the animal. In those animals wherein the dosage employed killed in 24 to 48 hours, the changes produced were analogous to early abscess formation *i. e.* predominant necrosis and peripheral polymorphonuclear neutrophilic cellular response. On the other hand the reduced dosage provoked changes conforming to those noted in miliary tuberculosis, namely, epithelioid cell proliferation, giant cell formation and peripheral lymphoid cell infiltration.

The results of our experiments show, as has the work of others, that experimental bronchomonoliasis can readily be produced in the rabbit by the intravenous injection of *Monilia albicans*. Furthermore the pathological features conform to the disease in man. We have shown further, however, that the histopathological changes produced in the rabbit present variants in the tissue reactions conforming regularly to the duration of the experimental disease as controlled by the dosage employed. Herein there is presented further analogy to the acute and chronic types as seen in man.

10745 P

Quantitative Prothrombin and Hippuric Acid Determinations as Sensitive Reflectors of Liver Damage in Humans.*

SLOAN J. WILSON. (Introduced by Charles A. Doan.)

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With the development of quantitative methods for the determination of plasma prothrombin by Quick¹ and Warner, Brinkhous and Smith,^{2,3} it has been established that the liver is an extremely important intermediary in the production of this important coagulation element. Smith, Warner and Brinkhous³ report a marked decrease in prothrombin after severe chloroform-induced liver damage in dogs. The extirpation of 60 to 75% of the liver in rats⁴ resulted in a temporary decrease in the plasma prothrombin to 30 or 40% of normal with a subsequent gradual return to normal during the 10 days to 3 weeks required for the regeneration of the liver to its normal weight.

In the present study observations have been made on normal human subjects and on patients with various degrees of liver damage without biliary obstruction or fistulae. The two-step method of Warner, Brinkhous and Smith was used to determine the level of the plasma prothrombin. The prothrombin, expressed in per cent of normal, was then compared with the plasma fibrinogen and the galactose, bromsulphalein and hippuric acid liver function tests.⁵

The quantitative level of the plasma prothrombin was found to correlate closely with the quantity of hippuric acid excreted. (See Chart 1). In those individuals with proven liver damage in which the hippuric acid excretion was 0.91 to 2.0 g, the prothrombin was found to be 19 to 37% of normal; with 2.0 to 3.9 g, the level was 33 to 90% of normal; and with 3.9 to 4.56 g hippuric acid excre-

* Aided by a grant from the Comly Research Fund, Ohio State University.

¹ Quick, A. J., Stanley-Brown, M., and Bancroft, F. W., *Am. J. Med. Sci.*, 1935, **190**, 501; Quick, A. J., *Am. J. Physiol.*, 1936, **114**, 252; Quick, A. J., *J. A. M. A.*, 1938, **110**, 1658.

² Warner, E. D., Brinkhous, K. M., and Smith, H. P., *Am. J. Physiol.*, 1936, **114**, 667.

³ Smith, H. P., Warner, E. D., and Brinkhous, K. M., *J. Exp. Med.*, 1937, **66**, 801.

⁴ Warner, E. D., *J. Exp. Med.*, 1938, **68**, 831.

⁵ Quick, A. J., *Proc. Soc. Exp. Biol. and Med.*, 1932, **29**, 12; *Am. J. Med. Sci.*, 1933, **185**, 630.

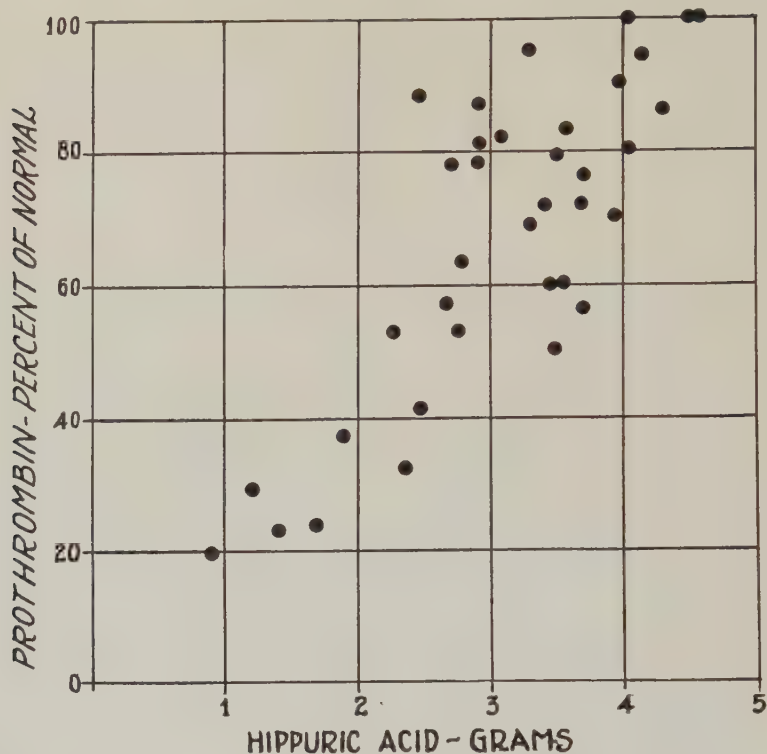


CHART 1.

Illustrating the close correlation of plasma prothrombin determination (ordinate) and the Quick hippuric acid test (abscissa) in terms of liver damage as measured by these respective tests. Any decrease in hippuric acid output was always accompanied by a proportionately diminished prothrombin level in the blood. Each point represents a different patient.

tion, the prothrombin level was 70 to 100% of normal. In 2 patients with severe cirrhosis of the liver and a consistently low plasma prothrombin level of 23 and 29% respectively, the hippuric acid excretion in one was only 1.2 g, in the other 1.9 g, and large amounts of vitamin K and bile salts were without demonstrable effect on the prothrombin.

There was no consistent relationship found between plasma prothrombin and plasma fibrinogen. In one of the patients with cirrhosis of the liver, a consistently low prothrombin and an initially low fibrinogen, the latter became elevated during an acute parotitis, the prothrombin remaining unchanged. In another subject with an aplastic anemia, oral sepsis, continuous high fever of 104°F. and acute hepatic damage, the prothrombin was only 30% of normal but the fibrinogen was discovered to be elevated to 0.531 g %. Smith,

Warner and Brinkhous³ noted a similar phenomenon during an episode of canine distemper which complicated the convalescence in one of their dogs following chloroform-induced hepatic damage.

The galactose tolerance test was within normal limits in all of the individuals studied. There was a wide variation in the results recorded for the bromsulphalein dye test.

Conclusion. In the human subjects here studied, without biliary obstruction of biliary fistulae, the quantitative levels of plasma prothrombin and the amounts of hippuric acid excreted following the ingestion of a known quantity of sodium benzoate would seem to have reflected most sensitively and consistently the degree of liver damage existing. There was no such suggestive correlation or relationship observed between these tests and the plasma fibrinogen levels, the bromsulphalein dye clearance or galactose utilization, either singly or collectively, when all were studied in the same patient.

10746

Determination of Ascorbic Acid in Feces. Its Excretion in Health and Disease.

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In studying the absorption of ascorbic acid from the gastrointestinal tract, we found it necessary to develop a method for the estimation of ascorbic acid in feces. Although reference to ascorbic acid in feces has been made by 2 authors,^{1, 2} no description of the method used was given in one case¹ and in the second,² only an abstract was available which referred to a colorimetric procedure. In the method to be described, the total indophenol reducing substances of the feces are estimated, an aliquot is then treated with an ascorbic acid oxidase, after which the reducing value is again determined. The difference represents the ascorbic acid present.

Feces upon excretion are immediately weighed to the nearest gram and transferred to a mortar in which they are rubbed to a homogeneous sludge with a minimum volume of freshly prepared 5%

¹ van Eeckelen, M., *Biochem. J.*, 1936, **30**, 2291.

² Ishibashi, T., *Acta Pædiat. japon*, 1937, **43**, 187. Abstracted by *Am. J. Dis. Child.*, 1937, **54**, 1101.

metaphosphoric acid solution. The sludge is transferred quantitatively to a suitable sized stoppered graduated cylinder (500 ml for 100 g stool) with sufficient 5% HPO_3 to give a suspension (Suspension A) in which 5 ml contain 1 g of original feces. In making a 24-hour assay, feces should be suitably marked with string. Stools passed at night are rubbed up with a known weight (usually 10 g) of powdered HPO_3 in an ointment jar, and then placed in a refrigerator for analysis in the morning.

Approximately 100 ml of suspension A (divided between two 50 ml centrifuge tubes) are centrifuged at moderate speed for 20 to 30 minutes. From the tubes 50 ml of supernatant fluid are pipetted into a 200 ml volumetric flask. Through this liquid is passed a vigorous stream of N_2 or CO_2 for 20 minutes to remove any trace of H_2S . (We do not depend upon a later treatment with lead acetate alone to remove all traces of H_2S .) At the end of this time, the aeration tube is rinsed and the solution diluted to the mark with 5% HPO_3 . Twenty ml of the resulting solution (Fluid B) are now equivalent to 1 g of the original feces.

Two or more 5 ml aliquots (for checks) of Fluid B are pipetted into 15 ml centrifuge tubes. To each are added 1 ml of glacial acetic acid and 5 ml of 12.5% lead acetate solution. (62.5 g anhydrous neutral $\text{Pb}(\text{C}_2\text{H}_3\text{O}_2)_2$, C.P. are dissolved in 350 ml of distilled water to which sufficient glacial acetic acid (5 to 15 ml) to cause solution is added. This is diluted to a volume of 500 ml.) The tubes are mixed well and centrifuged for 2 to 5 minutes. The meta-phosphates of lead adsorb most of the color. Any remaining turbidity can be dispelled by the further addition of 2-3 drops of 10% HPO_3 . Two ml aliquots are now transferred to small test tubes, and titrated by daylight to a distinct pink with a standardized 2:6-dichlorophenol-indophenol³ solution* delivered from a 5 ml micro burette (div. 0.01 ml). A No. 20 hypodermic needle fitted to a ground burette tip permits delivery of drops of not over 0.015 ml. The data from this titration represents *total indophenol reducing substances* (expressed as ascorbic acid).

To determine *non-ascorbic acid reducing substances*, pipette 2 or more 5 ml aliquots of Fluid B into 15 ml centrifuge tubes. To each, add sufficient 10% NaOH to bring the reaction to pH 5.5 to 6.0 as tested with nitrazine paper. Add to each tube 1 ml of cauliflower oxidase prepared according to the method of Hopkins and Morgan.⁴

³ Farmer, C. J., and Abt, A. F., *Proc. Soc. Exp. Biol. and Med.*, 1935, **32**, 1625.

* Hereinafter referred to as "standardized dye."

⁴ Hopkins, F. G., and Morgan, E. J., *Biochem. J.*, 1936, **30**, 1446.

Mix thoroughly and incubate in a water bath at 45-50°C for 30 minutes. At the end of this period add to each tube 1 ml of glacial acetic acid and 5 ml of lead acetate solution. Mix thoroughly and centrifuge. Two ml aliquots from respective tubes are titrated to the same end point as that obtained above. From these data are calculated the *non-ascorbic acid reducing substances* of the feces, expressed as milligrams of ascorbic acid.

Example of Procedure. 132 g of feces were made up to 660 ml with 5% HPO_3 (Suspension A). Fifty ml of supernatant liquid from suspension A were diluted to 200 ml with 5% HPO_3 (Fluid B). To each of three 5 ml aliquots taken for analysis, were added 1 ml glacial acetic acid, and 5 ml 12.5% lead acetate solution. After centrifugation, 2 ml portions of respective supernatant liquids were titrated, requiring an average of 0.66 ml "standardized dye". From this is calculated the total indophenol reduction.

To each of the 3 additional samples of Fluid B, 0.50 ml of 10% NaOH were required to adjust to pH 5.5-6.0. One ml oxidase was then added. After incubation, and clarifying with acetic acid and lead acetate, 2 ml aliquots required an average of 0.20 ml "standardized dye" for titration. From this is calculated the non-ascorbic acid reduction. One ml of "standardized dye" was equivalent to 0.020 mg ascorbic acid.

Calculations. To simplify calculations, the following equations are used.

- (1) Total reduction = $4.40 \times A \times B \times E$.
- (2) Non-ascorbic acid reduction = $0.40 \times B \times C \times D \times E$.
- (3) Ascorbic acid (mg) = total reduction - non-ascorbic acid reduction.

where

A = ml dye used for 2 ml aliquot before oxidase treatment.

B = total volume of suspension A in ml.

C = ml dye used for 2 ml aliquots after oxidase treatment.

D = total volume to which 5 ml of Fluid B is diluted after addition of NaOH, oxidase, acetic acid and lead acetate. It will vary depending upon the amount of NaOH required to adjust the pH.

E = ascorbic acid equivalent of 1 ml of dye.

In the above example: A = 0.66 ml; B = 660 ml; C = 0.20 ml; D = 12.5 ml; and E = 0.020.

Therefore:

Total reduction = $4.40 \times 0.66 \times 660 \times 0.020 = 38.33$ mg.

Non-ascorbic acid reduction = $0.40 \times 660 \times 0.20 \times 12.5 \times 0.20 = 13.20$ mg.

Ascorbic acid = $38.33 - 13.20 = 25.13$ mg.

Recoveries of Added Ascorbic Acid: In checking the method the following procedure was adopted: To known quantities of fecal suspension A, ascorbic acid† was added in varying amounts. Five ml samples were then carried through the procedure. Data from a series of analyses as given in Table I, indicate a recovery of 98.2%.

In a similar series in which the 1 ml glacial acetic acid was omitted, the recovery dropped to 92.3%. It should also be mentioned that complete destruction of ascorbic acid was obtained within 30 minutes by the cauliflower oxidase between pH 5.0 and 8.2. We, however, suggest adjustment to pH 5.5 to 6.0 because it was found that a destruction of the non-ascorbic acid reducing substances occurs above pH 6.5. As would be expected from the wealth of reducing bacteria in the large intestine, all of the ascorbic acid in the feces was found to be in the reduced form, thus eliminating a preliminary treatment with H_2S .

Effect of Diet upon Ascorbic Acid Excretion. With a method available for the determination of ascorbic acid in feces, it was possible for the first time to follow simultaneously the blood level and the urinary and fecal excretions after ingestion of varying amounts of ascorbic acid. Finger blood was removed from the fasting individual, and the plasma ascorbic acid content determined by the micro-method of Farmer and Abt.⁵ Urine samples collected during the day

TABLE I.
Recoveries of Added Ascorbic Acid.

Blank* as mg Ascorbic Acid	Ascorbic Acid Added, mg	Total Present, mg	Recovery, mg	% Recovery
.0456	.0252	.0708	.0690	94.6
.0154	.0348	.0402	.0474	94.4
.0456	.0378	.0834	.0854	102.4
.0310	.0378	.0688	.0676	98.3
.0176	.0382	.0558	.0520	93.2
.0384	.0482	.0866	.0852	98.4
.0456	.0504	.0960	.0976	101.7
.0154	.0524	.0678	.0680	100.3
.0342	.0544	.0886	.0904	102.0
.0374	.0690	.1084	.1036	95.6
.0154	.0698	.0852	.0870	102.4
.0176	.0764	.0940	.0900	95.8
.0176	.0954	.1130	.1104	97.7
				98.2 ± 0.6

* Blank = indophenol reducing substances other than ascorbic acid.

† We are indebted to Merck and Co., Inc., Rahway, N. J., for supplying the ascorbic acid (Cebione) used in this investigation.

⁵ Farmer, C. J., and Abt, A. F., *Proc. Soc. Exp. Biol. and Med.*, 1936, **34**, 146.

were acidified with metaphosphoric acid and titrated immediately. Night samples were preserved in the refrigerator in large bottles containing 10 g of metaphosphoric acid and titrated early the following morning. Fecal samples were analyzed immediately after excretion.

The subject consumed a normal diet which was relatively high in vitamin C. In addition, varying amounts of l-ascorbic acid were

TABLE II.
Comparative Studies of Blood Plasma Level, Urinary and Fecal Excretions, on
Various Levels of Ascorbic Acid Intake.
Subject H.C.

Date	Dietary Intake* mg Ascorbic Acid	Plasma Ascorbic Acid, mg %	Urinary Excretion		Fecal Excretion	
			Volume, ml	Excreted Ascorbic Acid, mg	Moist wt, g	Ascorbic Acid, mg
8/18	75				109.2	6.92
8/19	70	.88	1047	15.36	54.7	5.56
8/20	90	.68	810	16.63	31.4	4.35
8/21	35	.92	884	33.50	41.3	2.56
8/22	80	.92	1141	17.93	31.2	3.90
8/23	100	.96	847	30.34		
8/24	60	.76	602	16.78	125.2	15.44
Avg	73	.85	888	21.67	56.1	5.53
8/25	250	1.04	806	74.33	62.6	10.00
8/26	260	1.04	656	125.44	177.9	16.40
8/27	275	1.28	831	133.90	93.5	12.68
8/28	230	1.48	684	87.75		
8/29	260	1.76	825	155.53		
8/30	250	1.64	915	133.39		
8/31	250	1.76	829	123.94	169.2	29.60
Avg	255	1.43	792	119.18	71.9	9.81
9/1	540	2.04	867	252.61	70.2	12.70
9/2	570		810	180.45		
9/3	530	1.60	833	270.77	160.6	20.90
9/4	530	1.44	848	234.71		
9/5	550	1.44	1004	286.53	361.2	48.0
9/6	580	1.84	1012	454.91		
9/7	530	1.88	828	523.73	150.8	15.0
Avg	547	1.71	886	315.10	106.1	13.80
9/8	1050	1.68	1139	373.98	75.0	11.80
9/9	1070	1.60	932	379.86	140.0	16.16
9/10	1070	1.82	602	348.25		
9/11	1030	1.48	821	380.91		
9/12	1090	1.52	1266	482.14	149.9	33.31
9/13	1050	1.68	1212	497.79	149.0	18.12
9/14	1030	1.48	1367	375.90		
Avg	1054	1.61	1048	405.55	73.4	11.34

* Mg ascorbic acid in basal diet estimated from known food content, to which amount taken as supplement is added.

ingested. The daily supplement was taken in a single dose. To mark each day's feces, respectively, strings of a characteristic color were swallowed with each meal. Table II summarizes the results obtained on one of us (H.C.) during 28 consecutive days.

A relatively small but definite excretion into the feces can be noted in a normal individual, irrespective of intake. This is increased but slightly upon ingestion of large amounts of ascorbic acid. On the other hand, the blood plasma level rises with the intake until a maximum is reached, paralleled by a corresponding rise in the urinary excretion.

An average daily fecal excretion of 4.92 mg ascorbic acid was obtained in a study of 12 normal young male medical students subsisting on their usual mixed diet. A study of fecal excretion of patients suffering from various gastro-intestinal disorders showed much variation. Thus, an alcoholic following gastric resection excreted 233 mg when receiving 300 mg orally and 1000 mg intravenously per day. In a case of esophageal stricture, where 1000 mg were given intravenously, the daily fecal excretion averaged 1.5 mg over a period of 14 days. The excretion in colitis varied with the number and type of stool. One case receiving 450 mg ascorbic acid daily by mouth, excreted 34 mg into the feces while having 12 to 15 liquid stools per day. Another case in remission on a similar intake excreted 18 mg daily in 2 to 3 formed stools.

Conclusions. 1. A method for the estimation of ascorbic acid in feces is described, and by its use, the fecal content of the normal individual on an adequate but unsupplemented diet is shown to average about 5 mg daily. 2. The plasma, urinary and fecal ascorbic acid contents have been followed in a normal individual after administration of varying amounts of l-ascorbic acid by mouth. Large variations in the dietary intake were shown to affect the fecal excretion only slightly. 3. Patients suffering from certain gastro-intestinal disorders excreted larger quantities of ascorbic acid in the feces than normal individuals.

10747

Beta Cell Changes in Guinea Pig Pancreas in Relation to Blood Sugar Level.*

G. GOMORI, N. B. FRIEDMAN AND D. W. CALDWELL. (Introduced by P. R. Cannon.)

From the Departments of Pathology and Medicine, University of Chicago.

The *beta* cells of the pancreatic islets are believed to be the source of insulin,¹ and changes in these cells associated with altered carbohydrate metabolism were described even prior to the discovery of insulin. Experimental procedures reported and designed by several workers to stimulate the carbohydrate-metabolizing activities of the pancreas were found to result in degranulation and vacuolization of the *beta* cells of the islets.

Homans² and Allen³ noted degranulation of the *beta* cells in subtotally depancreatized cats and dogs. Miyairi⁴ described atrophic (degranulated?) *beta* cells in dogs and guinea pigs given glucose and adrenalin as compared with the *beta* cells of starved animals. Woerner⁵ found "exhaustion" of the *beta* granules in guinea pigs given intravenous glucose over long periods of time.

Several other observers described non-specific granule changes. Ssoblew⁶ noted the presence of relatively few fuchsinophile granules in dogs given carbohydrate and intravenous glucose as compared with starved dogs where abundant granules were seen. Marrassini⁷ found margination of fuchsinophile granules after administering carbohydrate to rabbits. Poll⁸ compared the islet cells of starved frogs and those of frogs given glucose or adrenalin, and in the latter the cell inclusions seen in the starved animals had disappeared.

O'Leary,⁹ studying the mouse pancreas *in vivo*, described disap-

* This work in part has been done under a grant from the Douglas Smith Foundation for Medical Research of the University of Chicago.

¹ Best, C. H., and Taylor, N. B., *The Physiological Basis of Medical Practice*, Baltimore, 1937, William Wood and Company.

² Homans, J., *J. Med. Research*, 1914, **30**, 49.

³ Allen, F. M., *J. Metab. Research*, 1922, **1**, 5.

⁴ Miyairi, S., *Proc. Imp. Acad. Jap.*, 1927, **3**, 702.

⁵ Woerner, C. A., *Anat. Rec.*, 1938, **71**, 1.

⁶ Ssoblew, L. W., *Virchow Arch. f. path. Anat. u. Physiol. u. f. kl. Med.*, 1902, **168**, 91.

⁷ Marrassini, A., *Arch. Ital. de Biol.*, 1907, **48**, 369.

⁸ Poll, H., *Ergänzungsheft z. Anatomischen Anzeiger*, 1931, 71.

⁹ O'Leary, J. L., *Anat. Rec.*, 1930, **45**, 27.

pearance and presumable emptying of formed vacuoles in islet cells following the administration of glucose.

Vacuolization was noted by Ssobolew,⁶ Homans,² and Allen³ in the pancreatic remnants of glycosuric subtotally depancreatized cats and dogs, and these changes were taken to indicate functional overstrain of the islet cells. Kolossow¹⁰ found vacuolization in the *beta* cells of tritons after glucose administration.

The present experiments consisted of observations on the course of degranulation of the *beta* cells following the administration of a single dose of glucose.

Young guinea pigs (not fasted) weighing 300 to 500 g, were given intraperitoneally 2 g per kilo of glucose in a 10% aqueous solution. The blood sugar, determined by the Hagedorn-Jensen method on 0.1 cc of blood obtained from the ear, was followed throughout the experimental period.

Animals were sacrificed at various time intervals from the start of

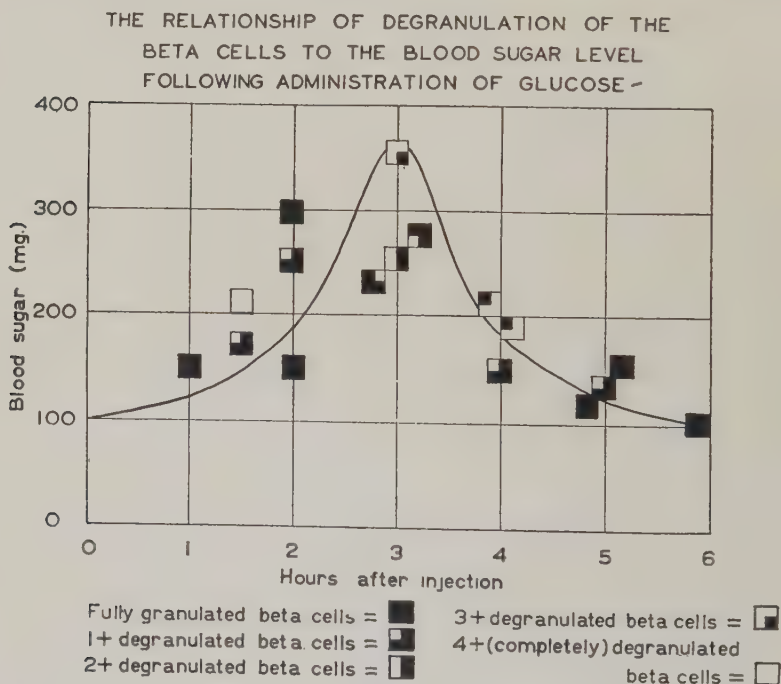


FIG. 1.

Each square represents a single animal and the degree of degranulation is indicated by the shading. The squares are plotted against the blood sugar level at varying times after the injection of glucose.

¹⁰ Kolossow, N. G., *Z. f. Mikro. Anat. Forsch.*, 1927, **11**, 43.

the experiment. Pieces of pancreas were fixed in Bouin's fluid with 2.5% sulpho-salicylic acid and in formol-Zenker. Paraffin sections were stained with the Mallory-Heidenhain azan stain and with chromium hematoxylin and phloxin after permanganate oxidation.¹¹

The sugar tolerance curves obtained showed a 250-350 mg peak at approximately 3 hours with a return to normal levels of about 100 mg at the 5th and 6th hours.

Some degree of degranulation of the *beta* cells was found in most of the animals whose blood sugar was elevated. This ranged from sparsity of granules in some of the *beta* cells or margination to complete absence of the granules in nearly all of the *beta* cells. The *alpha* cells appeared unaltered. These changes were uniform throughout the pancreas.

The most marked degranulation was present in those animals killed at the 3rd and 4th hours when the blood sugar was at its peak or was falling. Lesser degrees of degranulation were observed at the earlier and later observation periods. In both fed and fasted control animals with normal blood sugars, degranulation was not observed.

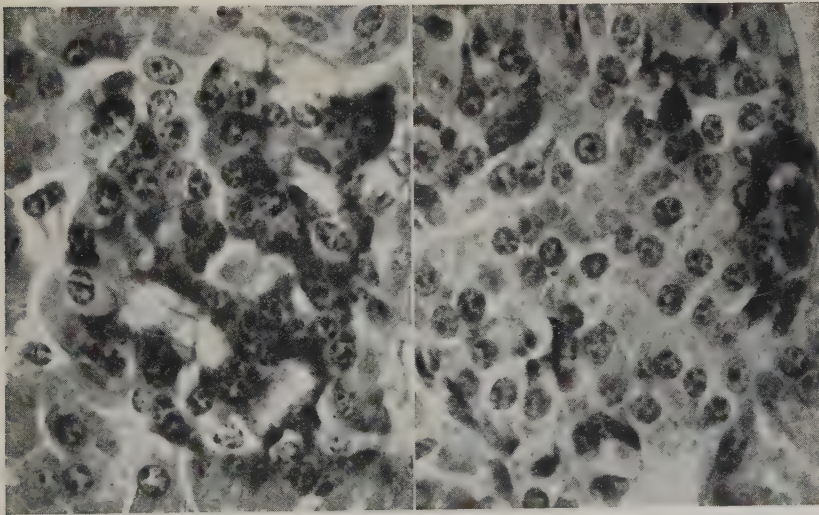


Fig. 2A (Left).

Microphotograph of a normal islet. The dark cells are *beta* cells laden with granules.

Fig. 2B (Right).

Microphotograph of a considerably degranulated islet. There are normally granulated *beta* cells along the edges whereas centrally located is a large group of degranulated *beta* cells.

¹¹ Gomori, G., *Am. J. Path.*, in press.

The granule changes were graded from 1+ to 4+ and are shown in relation to the blood sugar curve in Fig. 1. Examples of a well granulated islet and of a considerably degranulated islet (graded at 3+) are shown in Fig. 2.

This presumable cycle of disappearance and reappearance of the *beta* granules was confirmed by means of serial pancreatic biopsies in individual animals. These guinea pigs were given glucose subcutaneously and the abdomen opened at intervals under local novocaine anesthesia.

Conclusions. Disappearance of the specific granules of the *beta* cells in the pancreatic islets of guinea pigs given intraperitoneal glucose was noted during the subsequent hyperglycemia. The *beta* granules were again in evidence after the blood sugar reached normal levels. These histological changes, suggesting functional activity of the *beta* cells, may be related to the secretion of insulin in response to elevation of the blood sugar.

10748 P

Production of Fatty Livers in Guinea Pigs with Scorbuto-genic Diets.

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In the course of observations of Vitamin C deprivation in guinea pigs we noted that these animals showed at post-mortem large fatty livers in addition to the usual findings of scurvy. The pathology of the internal organs in scurvy has apparently not been extensively studied. Bessey, Menten, and King¹ have noted changes in the adrenal glands in which a decrease or disappearance of lipid material was observed. They also mentioned fatty changes in various organs including the heart, liver and skeletal muscles.

Twenty-five of 27 animals dying from scurvy on a modified Sherman *et al.*² scorbuto-genic diet showed moderately severe to very severe fatty changes in the liver both grossly and microscopically.

¹ Bessey, O. M., Menten, M. L., King, C. G., *Proc. Soc. Exp. Biol. and Med.*, 1934, **31**, 455.

² Sherman, H. C., La Mer, V. K., and Campbell, H. L., *J. Am. Chem. Soc.*, 1922, **44**, 165.

TABLE I.
Fat Analysis of Liver Tissue.

Serial No. of animal	Diet		Vitamin C		Total wt of liver, g	Body wt	% liver of body wt	% lipids in liver tissue
		No. days		No. days				
SM3	Starved	11	Abund.		10.5	252	4.1	1.4
NM 1	Normal		"					2.1
NM 3	"		"					2.0
NM 2	"		"		19.5	366	5.3	2.4
IM 1	No. 1	47	Free	25	30.3	289	10.5	23.9
IM 2	No. 1	50	"	28	30.5	328	9.3	26.1
IM 4	No. 1	49	"	27	22.0	275	8.0	10.9
IM 5	No. 1	50	"	28	26.0	335	7.7	11.8
IM 6	No. 1	52	"	30	18.0	259	6.9	12.2
HM 1	No. 1	54	Low	54	29.0	339	8.6	20.7
HM 2	No. 1	33	"	33		286		13.1
HF 3	No. 1	53	"	53	15.0	316	4.7	6.4
HF 4	No. 1	67	"	67	8.5	168	5.06	9.1
HF 1	No. 1	117	"	117	14.0	219	6.8	30.1
GM 2	No. 2	46	Free	21	13.0	172	7.5	11.7
GF 3	No. 2	46	"	21	13.5	194	6.9	10.4
EF 2	No. 2	100	Abund.		30.5	628	4.8	7.5
EM 1	No. 2	154	"		44.0	397	11.3	23.8

Diet No. 1—Rolled oats and wheat bran 59 parts, skimmed, dried powdered milk 30 parts, butter fat 8 parts, cod liver oil 2 parts, NaCl 1 part.

Diet No. 2—Rolled oats and wheat bran 48 parts, dextrose 10 parts, desiccated brewer's yeast 1 part, the rest as in diet No. 1.

Five of these were checked chemically by the Van Slyke³ gasometric method and showed a fat content of 11 to 26%. Another group of 5 guinea pigs placed on this diet but given subminimal amounts of pure ascorbic acid, to prolong the period of survival, showed a fat content of 6 to 30%. The animal having 30% fat in the liver had the longest period of survival (Table). The subcutaneous tissue, mucous membranes, and liver of this animal had a uniform greenish color, showing that jaundice was present before death.

To find out if other factors beside the Vitamin C deficiency were operating in this experiment, we starved 4 animals, giving them only Vitamin C and water. None of these showed gross or microscopic evidence of fatty changes, and the content of fat in the liver of one of those, determined chemically, was 1.4%.

Five other animals were placed on a further modified basal diet enriched with dextrose and desiccated yeast (Diet No. 2, Table). All of these dying from scurvy showed definite fatty changes; in 2 of these this was substantiated by chemical analysis. The degree of fatty changes in this group was probably smaller than in the animals on the unmodified diet No. 1 (Table).

Two animals each were placed on diets No. 1 and No. 2 plus an abundance of ascorbic acid for a minimum of 3 months. These animals seemed to thrive up to about 3 months and then began to lose weight and look ill. Three of these animals were sacrificed at this crucial period and showed moderate degrees of fatty degeneration. EF2 (Table) whose liver was analyzed chemically showed 7.5% fat. The 2 animals on the No. 1 diet showed histologically about the same or slightly greater fatty change. One of these animals (EM1) was permitted to live on. He continued the downhill course and died at the end of 154 days showing a large, firm, nodular liver, ascites with 25 cc of fluid in the peritoneal cavity and edema of the abdominal wall.

Summary and Conclusions. Scurvy produced in guinea pigs by the scorbutogenic diets used is accompanied by severe fatty degeneration of the liver. This process is slightly retarded by additional carbohydrate in the diet. These diets are apparently deficient in some other factor or factors, whose presence is necessary for normal liver physiology and morphology.

³ Kirk, E., Page, I. H., and Van Slyke, D. D., *J. Biol. Chem.*, 1934, **103**, 203.

10749 P

Growth and Viability of *Cryptococcus hominis* at Mouse and Rabbit Body Temperatures.

L. R. KUHN. (Introduced by G. M. Dack.)

From the Department of Bacteriology and Parasitology, University of Chicago.

Mice were readily infected with strains of *Cryptococcus hominis* isolated from human cases of torula meningitis, subcutaneous tumor, and generalized infection.¹ Death occurred following a regular increase in the number of viable yeasts in the mouse tissues, especially in the brain and lungs. On the other hand, rabbits resisted infection and the number of viable yeasts in the tissues decreased regularly, even when very large doses of cryptococci were given intravenously. Mice and rabbits each were found to produce only a very low grade acute cellular reaction when the yeasts were injected intraperitoneally.²

When the normal body temperatures* of mice were compared with those of rabbits and especially when it was noted that mice failed to produce fever with experimental cryptococcus infection, whereas the temperatures of injected rabbits readily rose to 105°-107°F (40.6-41.7°C), the writer decided to determine if the growth and viability of *C. hominis* at rabbit and mouse body temperatures was significantly different.

Six strains of group III cryptococci (Benham grouping³) which were isolated from human cryptococcus infections during the past 5 years were inoculated into dextrose veal infusion broth (pH 7.0) and grown at 99°F (37.3°C), 101°F (38.3°C), 103°F (39.4°C), 105°F (40.6°C), and 107°F (41.7°C). At the higher temperatures fewer organisms were produced and viable cells (determined by plating in Sabouraud's dextrose agar) decreased in number. The effect on the number of viable cells of one of the strains of *C. hominis* is shown in Table I.

At 105°F (40.6°C) viable cells had decreased in number after 3

¹ Report in preparation.

² Kuhn, L. R., *Arch. Path.*, 1939, **27**, 803.

* The mean of 546 recordings of the body temperatures of normal mice was 99.1°F. The temperatures ranged from 95.5° to 101.5°, with 75% of the recordings between 98° and 100.5°. The mean of 334 recordings of the body temperatures of normal rabbits was 103.15°F. The temperatures ranged from 102.25° to 104.25° with 87% of the recordings between 102.75° and 103.5°.

³ Benham, R. W., *J. Inf. Dis.*, 1935, **57**, 255.

TABLE I.
Effect of Temperatures Between 99°F (37.3°C) and 107°F (41.7°C) on Number of Viable Cryptococci in Broth Cultures.

Time in days after inoculation	Mean* of the number of viable yeasts per mm ³ in 3 tubes incubated at each temperature				
	99°F† 37.3°C	101°F† 38.3°C	103°F† 39.4°C	105°F† 40.6°C	107°F† 41.7°C
0	570	630	583	567	593
½	1713	1173	650	280	45
1	2707	2163	723	151	13
2	4033	2767	667	42	2
3	4943	2620	458	5.6	0.04
4	5680	2253	246	0.80	0.0036
5	6263	1750	87	0.03	0†-0.001
6	6723	643	15	0.0014	0
7	7267	224	2	0	0
8	7480	58	0.09	0	0

Each tube contained 5 cc of dextrose veal infusion broth and was inoculated from a similar 24-hour broth culture.

* The greatest variation from the mean in any one of the 3 tubes was 5% of the mean.

† The temperature of the water bath varied 0.25°F above and below the temperature given.

‡ Two tubes were sterile, the third had 1 viable yeast per cc of broth.

hours' incubation, at 107°F (41.7°C) they decreased within an hour and only 2.5% survived at the end of 24 hours.

The total number of yeast cells was also counted (hemacytometer) in tubes of dextrose veal infusion broth and Sabouraud's dextrose broth incubated at 22°, 29° and 36°C. With each strain of cryptococcus employed samples examined from 1 to 5 days after inoculation showed that the cell count was higher in the tubes incubated at 29°C than in the 22° or 36°C tubes, and higher in the 22°C than in those incubated at 36°C. The optimum temperature for these organisms appears, therefore, to be below the normal body temperature of either the mouse or the rabbit.

In attempting to account for the resistance of the rabbit to experimental cryptococcus infection, the high normal and fever temperatures of this animal must be considered. Experiments on the effect of alteration of body temperature of experimental animals on their resistance to these organisms are now in progress.

10750 P

Periodicity of Sporadic Bacillemia in Experimental Tuberculosis in Dogs.

JOHN S. HOWE. (Introduced by A. J. Nedzel.)

From the Department of Pathology, Bacteriology and Public Health, University of Illinois, College of Medicine, Chicago, Illinois.

The author¹ has reported the occurrence of sporadic bacillemia in 17 of 20 dogs infected experimentally with virulent human tubercle bacilli (H 37). This report represents a further study of some factors in the time relationships of bacillemia in tuberculous dogs, including some of the animals included in the previous report.

Dogs were injected intravenously or subcutaneously with virulent human tubercle bacilli (H 37) in a suspension of Kaolan, mineral oil, and normal saline, the doses ranging from 3 to 20 mg. Femoral arterial punctures were performed daily on all dogs, and 3 to 5 cc of arterial blood withdrawn, under sterile precautions and injected directly into guinea pigs, usually subcutaneously in the right inguinal region. Endermal tuberculin tests were done on all guinea pigs before inoculation with the dogs' blood, and at intervals up to 3 months after inoculation, when all were sacrificed and necropsied.

The presence of tuberculosis was established on the basis of the characteristic pathology of injection—tuberculosis, supported by positive tuberculin reactions and microscopic examination of smears for tubercle bacilli.

All guinea pigs injected on 2 successive days were usually kept in the same cage during the 3-month period of observation. At necropsy it was noted that in some cages there was a high percentage of guinea pigs positive for tuberculosis while in other cages all were negative. The possibility that this might be due to spontaneous infection was considered. However, as Saenz *et al.*² have pointed out, spontaneous laboratory infection does not occur in less than 6 months' exposure, and the longest period of exposure to infection possible in this experiment was 3 months. Moreover, the anatomical distribution of the tuberculous lesions in our guinea pigs conformed closely to those described by Wilson³ as typical of in-

¹ Howe, J. S., *PROC. SOC. EXP. BIOL. AND MED.*, 1939, **40**, 509.

² Saenz, M., Costil, L., and Sadettin, M., *Compt. rend. Soc. de Biol.*, 1935, **119**, 266.

³ Wilson, *Tuberculous Bacillemia*, London, 1933.

jection—tuberculosis *i. e.*, inguinal glands and spleen chiefly involved, rarely liver, very rarely lungs or intestines.

From these considerations it seemed that spontaneous infection could be excluded. Furthermore the possibility that errors in technic might play a part seemed unlikely, since the blood was drawn daily under sterile conditions and injected directly into guinea pigs which were then carefully identified and segregated.

Since technical errors and spontaneous infection could be eliminated as causes, this apparent periodicity of bacillema in animals observed at the same period seemed to justify further study.

Charts were made for each dog showing the occurrence and relative amount of bacillema on each day of the observations. Charts of all dogs observed over the same period of time were then superimposed to show any periods of bacillema occurring in several or all of the dogs. Protocols for 3 such periods of observation, representing the major part of the experiment, are appended.

These protocols confirm the tendency to periodicity of bacillema in dogs observed over the same period of time. This periodicity seems not dependent on the duration of the infection, for it is evident in animals inoculated at different times but observed during the same experiment. It seems to be unrelated to the outcome of the infection, for it is evident in comparing the charts for animals that recovered with those that died. It seems independent of the anatomical distribution of the tuberculous lesions, as is evident in comparing the charts of dogs injected intravenously with those injected subcutaneously.

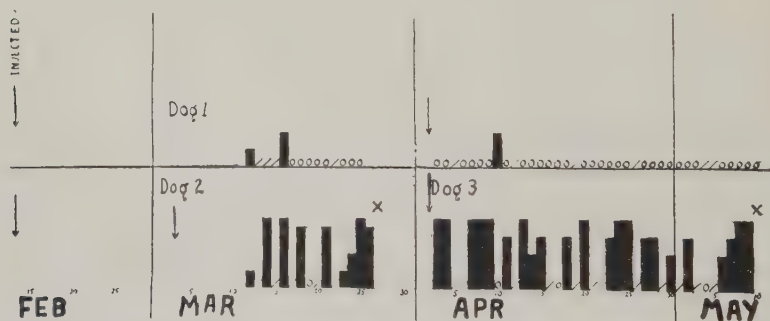


FIG. 1.

Each small square represents one day. Day of inoculation is indicated by arrow. Solid black columns denote bacillema, the height of column roughly indicating the degree of bacillema. Negative days indicated by 0. Diagonal line represents days on which no blood was drawn.

Dog No. 1 was injected subcutaneously. Dogs 2 and 3 were injected intravenously. Note the close correspondence of periods of bacillema.

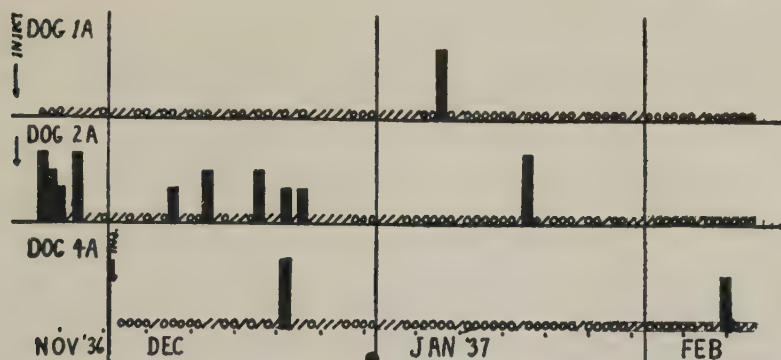


FIG. 2.

Dogs 1A and 2A injected intravenously. Dog 4A injected subcutaneously. Less marked similarity of charts, but 2A and 4A correspond on December 21st.

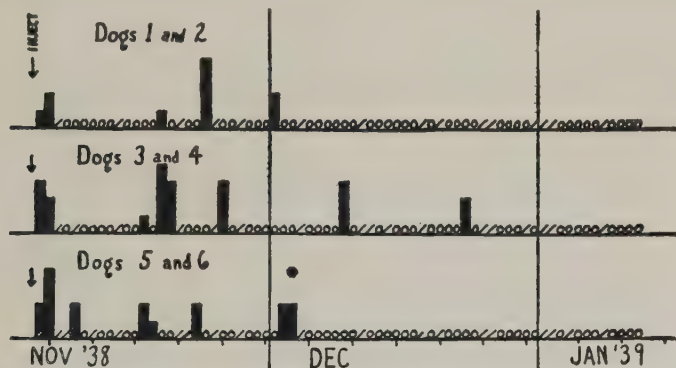


FIG. 3.

All dogs injected intravenously. Blood from each pair of dogs (1 and 2, etc.) pooled before injection. Periods of bacillema are strikingly similar in the three groups of dogs.

Search of the literature reveals no mention of periodicity in bacillema, although certain clinical findings might well suggest the possibility. The explanation of this periodic bacillema, simultaneous or closely following in several dogs, must lie in some factor which tends to affect all the animals in the same manner at about the same time. All dogs were on standard diets and under good laboratory conditions. Further study of various environmental factors in relation to this periodic bacillema is in progress.

Electrokinesis in Endolymph as Possible Cause of "Galvanic" Falling and Past Pointing.

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There are a number of differences between the response to the passage of electrical currents through the head and the response of nerves to electrical stimulation. In the former cases responses occur only upon the continuous passage of current and not on the make and break. In the latter the response follows the transient not steady state of current. In the former alternating current or faradic current produces no response, in the latter they are effective stimuli. In the former the direction of response is related to the direction of current, not in the latter. Since stimuli of a duration of .000033 of a second at 15000 stimuli a second were effective to produce a response, if the vestibular nerve were being stimulated directly it would indicate that the irritability of the vestibular nerve was as great or greater than that of a motor nerve, which is unlikely.

Because of these observations we believe that the passage of a current through the head does not stimulate the vestibular nerve or its connections directly to produce falling or past pointing.

It is suggested that some physical change in the endolymph is produced by the passage of current through the head. Using rectangular shaped unidirectional current of variable periods as stimuli, it was found that when the interval was 1 to 1, falling occurred at 2.6 milliamperes, when 1 to 3, at 1.39 milliamperes, and 1 to 7 at 0.71 of a milliampere. It is pointed out that this change is not the production of heat or electrolysis since the current per unit is not a constant in producing falling or past pointing.

Using repetitive condenser discharges as the stimulus, we found

TABLE I.

Voltage	Stimuli, per sec	Duration of stimuli, sec	Duration of interval, sec
120	22.3	.0027	.041
120	19.5	.0044	.050
120	16.4	.0096	.051
120	13.4	.014	.060
120	10.4	.024	.072—No falling

TABLE II.

Voltage	Stimuli, per sec	Duration of stimuli, sec	Duration of interval, sec
57	28.3	.004	.035
65	25.4	.004	.035
74	22.3	.004	.044
76	19.5	.004	.051

that to produce falling or past pointing, when the voltage at which the condensers were being charged was kept constant as the duration of the intervals between stimuli was increased by diminishing the frequency of stimuli, it was necessary to increase the duration of the stimulus.

When the duration of the stimulus was kept constant and the duration of the intervals lengthened, it was necessary to increase the voltage to produce falling or past pointing.

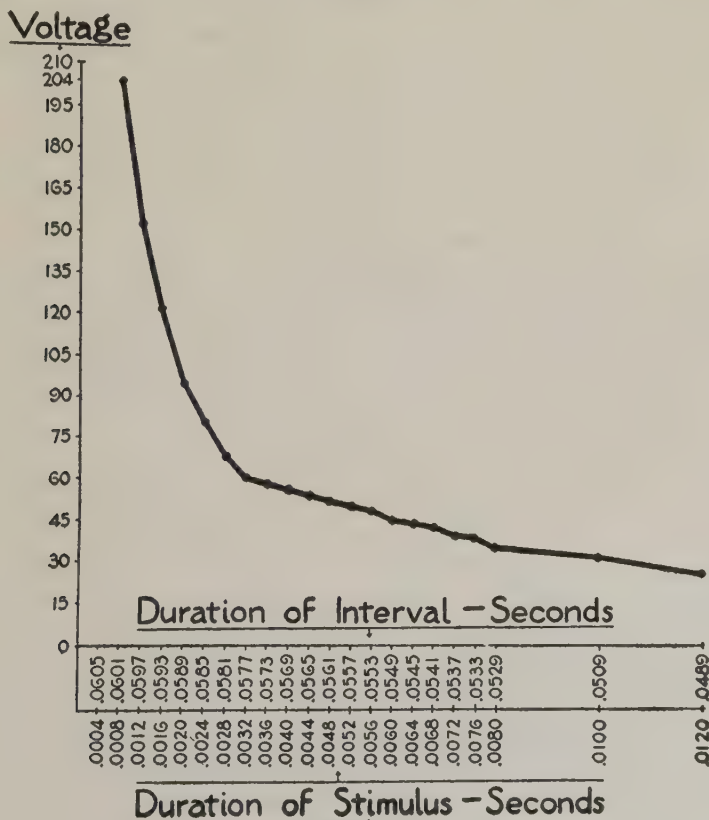


FIG. 1.

When the duration of stimulus was diminished by using condensers of lesser capacities and at the same time the duration of the interval increased, the frequency remaining constant, it was necessary to increase the voltage at which condensers were charged to produce falling or past pointing. (Figure 1.)

Because relationships of current, duration of stimuli and interval between stimuli, similar to those described in electrokinetic phenomena are also found in the production of falling or past pointing when a galvanic current is passed through the head, it is suggested that an electrokinetic change in the endolymph is responsible for the stimulation of sensory end organs, when a galvanic current is passed through the head.

10752 P

Normal Development and Regression of the Prostate Gland of the Female Rat.*

DOROTHY PRICE. (Introduced by Carl R. Moore.)

From Hull Zoological Laboratory, the University of Chicago.

In an earlier paper,¹ the normal embryological and post-natal development of the prostate gland of the male rat was described, and brief mention was made of the embryology of the female homologue. In young males, castrated at birth, the prostate continues to differentiate in the absence of the testes and maintains this differentiation for a limited period of time. The normal post-natal development of the female homologue has now been studied for comparison with the male and a portion of these data will be presented here.

Marx^{2, 3} first described prostatic lobes in female rats and Korenchevsky^{4, 5, 6} showed that female prostates, hypertrophied by androgens, were histologically identical with the ventral lobes of males. Estrogens produced no stimulation. Androgenic stimulation and

* This investigation has been aided by grants to the University of Chicago from the Rockefeller Foundation.

¹ Price, D., *Am. J. Anat.*, 1936, **60**, 79.

² Marx, L., *Arch. Entw.-mech.*, 1931, **124**, 584.

³ Marx, L., *Z. Zellforsch mikroskop. Anat.*, 1932, **16**, 48.

⁴ Korenchevsky, V., *Nature*, 1935, **136**, 185.

⁵ Korenchevsky, V., and Dennison, M., *J. Path. Bact.*, 1936, **42**, 91.

⁶ Korenchevsky, V., *J. Physiol.*, 1937, **90**, 371.

lack of estrogenic effects have been reported by other workers.^{7, 8} Witschi and collaborators,^{9, 10} likewise noted that female prostates reacted in the same way to injected sex hormones as the male ventral lobe, and by selective breeding have greatly increased the incidence of prostates in females. Female prostates have been induced in embryos of rats and mice by injection of pregnant females with androgenic hormones.^{11, 12, 13}

The female prostate has been described as atrophic or rudimentary and the occasional macroscopically visible lobes have been considered similar histologically to the atrophied castrate male prostate. The light areas which are so typical of the normal secreting ventral prostate of the male have never been described in the female except in rats treated with androgenic substances.

The cases presented are limited to macroscopically visible lobes and the number is small due to the low incidence (under 2%) of such prostates in our rat colony, but the age range is wide and an adequate comparison of the female gland with that of the male can be made. Table I lists 19 prostates in rats ranging in age from 5 days to adult. At 5 days the acini are small solid cords of cells which develop lumina by 15 days and epithelial light areas, diagnostic of secretory activity, at 21 days. Light areas develop in ventral prostate lobes of normal males at 12 days and in males castrated

TABLE I.
Occurrence of Light Areas in Prostate Glands of Untreated Female Rats.

Age in days	Position of lobes	Light areas	Age in days	Position of lobes	Light areas
5	Right	0	32	Right	+
15	Left	0	37	"	+
17	Right	0	40	"	+
21	"	+	40	"	+
21	Bilateral	+	45	"	0
28	Right	0	61	"	0
28	Bilateral	R +	80	"	0
		L 0	ca. 100	"	0
32	Right	+	ca. 100	"	0
32	"	0			
32	Left	+			

⁷ Hamilton, J. B., and Wolfe, J. M., *PROC. SOC. EXP. BIOL. AND MED.*, 1937, **36**, 465.

⁸ Deanesly, R., and Parkes, A. S., *Lancet*, 1938, Sept. 10, 606.

⁹ Witschi, E., Mahoney, J. J., and Riley, G. M., *Biol. Zent.*, 1938, **58**, 30.

¹⁰ Witschi, E., Riley, G. M., and Gardner, M., *Genetics*, 1939, **24**, 90.

¹¹ Greene, R. R., and Ivy, A. C., *Science*, 1937, **86**, 200.

¹² Greene, R. R., Burrill, M. W., and Ivy, A. C., *PROC. SOC. EXP. BIOL. AND MED.*, 1938, **38**, 1.

¹³ Raynaud, A., *Bull. Biol. France et Belg.*, 1938, **72**, 297.

at birth at about 17 days. Light areas appeared in 9 out of 11 female prostates between the ages of 21 and 40 days. The degree of development of the gland varied greatly as it does in prostates of young male castrates. After 40 days of age the female prostate begins to regress as does that of the young male castrate, although the histological picture is not so extreme, in many cases, as in the adult male castrated for twenty days. From these data it appears that the prostate gland of a certain proportion of female rats undergoes normal development, differentiation and regression comparable to that of the prostate of the young castrated male.

Howard^{14, 15} suggested that the temporary development of the prostate of the young castrate male rat might be attributed to andromimetic activity of the young adrenal cortex. Prostate development and regression coincided, respectively, with a well differentiated juvenile cortex and one which had lost its characteristics and approached the adult condition. Davidson and Moon¹⁶ found gross and histological stimulation of the prostate in young castrated male rats after administration of adrenocorticotrophic hormone which caused hypertrophy of the adrenal cortex. Burrill and Greene¹⁷ compared prostates of young male castrates with those of adrenalectomized rats and with those from rats both adrenalectomized and castrated. Their results strongly suggest that the adrenals of young castrates maintain the prostates in a functional state.

The present findings indicate that whatever is responsible for temporary prostate differentiation in young castrated males may be operating for a like period in young normal females. The juvenile adrenal cortex is the probable factor and this is supported by Howard's studies on the rat adrenal which showed that in both males and females the juvenile cortex is differentiated at three weeks of age and has lost its distinctive character by about 40 days.

Grafts of male prostate tissue have been kept in a functional state with light areas for months in normal adult female hosts.¹⁸ This indicates the presence of some androgenic substance in the adult female but this is probably of ovarian origin. The difference in threshold response between male and female prostates (unpublished

¹⁴ Howard, E., *Am. J. Physiol.*, 1937, **119**, 339.

¹⁵ Howard, E., *Am. J. Anat.*, 1938, **62**, 351.

¹⁶ Davidson, C. S., and Moon, H. D., *PROC. SOC. EXP. BIOL. AND MED.*, 1936, **35**, 281.

¹⁷ Burrill, M. W., and Greene, R. R., *PROC. SOC. EXP. BIOL. AND MED.*, 1939, **40**, 327.

¹⁸ Price, D., *Anat. Rec.*, 1937, **70**, suppl. 1, 60.

results) may account for the absence of light areas in adult female prostates.

Conclusions. In some young normal female rats, as in young castrated males, the prostate grows, develops large acini, high epithelium and light areas diagnostic of a secretory state. In both, the prostate differentiation is maintained only until about 40 days when regression begins. The factor causing this temporary differentiation is thought to be the juvenile cortex of the adrenal.

10753 P

Delayed Prothrombin Clotting Time in Avitaminosis A and Pellagra-Like Chicks.

HERMAN C. MASON AND MARGARET E. SMITH. (Introduced by Lloyd Arnold.)

From the Department of Bacteriology and Public Health, University of Illinois College of Medicine, Chicago, Ill.

A study is being made of the virus-neutralizing properties of fowl blood, plasma and serum, and the relation of refractoriness to prothrombin clotting time in avitaminosis.

Dam, Schønheyder, Tage-Hansen,¹ Almquist,² and Quick³ have shown that prothrombin deficiency can be detected in chicks on a depleted vitamin K diet at an early date.

The experiments herein reported were designed to test prothrombin clotting time on chicks depleted of vitamin K,² vitamin A,⁴ and of chicks kept on the pellagra-like "F" ration of Ringrose, Norris and Heuser.⁵ The chicks used in these experiments came from the same parent stock and received rations prepared bi-weekly. Several control series of chicks in the past showed that depletion of vitamin A occurred between the 7th and 14th days with an average weight of 31-32 g at death. The chicks on the pellagra-like "F" ration were in good health and weighed 61.2 g. To produce depletion 60 day-old chicks were placed on each vitamin deficiency level making a total of 180 chicks. Only the K free chicks were kept from their

¹ Dam, H., Schønheyder, F., Tage-Hansen, E., *Biochem. J.*, 1936, **30**, 1075.

² Almquist, H. J., *J. Biol. Chem.*, 1936, **114**, 241.

³ Quick, A. J., *Am. J. Physiol.*, 1937, **118**, 260.

⁴ Emmett, A., and Peacock, G., *J. Biol. Chem.*, 1923, **56**, 679.

⁵ Ringrose, A., Norris, L., and Heuser, *Poultry Sci.*, 1930-31, **10**, 166.

feces. The normal controls were kept on a chick start ration plus milk ad lib.

For the estimation of prothrombin in chick plasma Quick's⁶ wet and dry methods were employed. 1.5 cc of blood drawn from the heart was used in each determination. The clotting time was read by means of an interval timer and the beginning of the clot taken as prothrombin time. The dry method exhibits more variation in its activity than the wet confirming Quick. The values obtained by Quick and by us allow no quantitative estimation or at best were roughly so for vitamin K free chicks. Our shortest clotting time on normal controls was 5-13 sec. in contrast to 10-11 sec. obtained by Quick.

The results indicate clearly with both wet and dry methods that the normal clotting time may vary, with the average time ranging from 12 to 40 sec. Thirty determinations on 15 ten-day-old chicks depleted of vitamin K gave prothrombin clotting times over 50 sec. and longer. Twenty-six determinations on 13 ten-day chicks, and 25 determinations on 7 thirteen-day chicks maintained on the vitamin A deficiency ration gave prothrombin clotting values over 5 minutes and longer. Twenty-eight determinations on 14 eleven-day chicks kept on the pellagra-like "F" ration gave prothrombin clotting values over 5 minutes with but one chick exhibiting a clotting time of 25 sec. with both methods. Occasionally normal control chicks sustained on standard commercial rations may give prothrombin clotting values over several minutes.

At present no curve for prothrombin values based on Quick's results and ours can be considered reliable in the chick, or as he states comparable to the rabbit. Further we can find no relation between blood clotting time and prothrombin clotting time. If one attempts to plot the relation of the clotting time of recalcified chick plasma to the concentration of prothrombin in plasma, the results yield exceedingly slight values to infinity for the prothrombin concentration. Quick's statement that prolongation of the clotting time to 45-50 sec. corresponds to a prothrombin concentration of less than 10% of normal with a distinct hemorrhagic tendency in vitamin K depleted chicks raises several questions in the depleted vitamin A and pellagra-like "F" ration chicks, because of the occurrence of prothrombin clotting times over 50 sec. and longer without the hemorrhagic tendency manifesting itself. The studies of Ringrose,⁵ Emmett⁴ and Cruikshank⁷ do not record the presence of a hemorrhagic

⁶ Quick, A. J., *Am. J. Physiol.*, 1935-36, **114**, 285; *J. A. M. A.*, 1938, **110**, 1658.

⁷ Cruikshank, E. M., *Nut. Abs. Rev.*, 1935-36, **5**, 2.

tendency associated with the other severe changes in any of the chicks maintained on their numerous deficient rations. Furthermore, the delay in prothrombin clotting time reported by us is not associated with a spontaneous hemorrhagic tendency to date. The blood clotting time was within normal limits for the vitamin A deficient and pellagra-like chicks. What relation these deficiencies have with the statement of Almquist and Stokstad⁸ that spontaneous recovery occurs in vitamin K depleted birds as they grow older is not yet clear.

At the present time the presence of anemia is being investigated. Also other vitamin deficiencies are being studied and their rôle on the prothrombin clotting time. Vitamin K assays on the avitaminosis A and pellagra-like chicks are being carried on.

Summary. Chicks receiving avitaminosis A and pellagra-like "F" rations failed to show normal prothrombin coagulation time. The plasma prothrombin clotting time was greatly delayed in these avitaminotic chicks without the distinct hemorrhagic tendency of a vitamin K depletion manifesting itself.

10754

Effect of Chemical Irritation of a Venous Segment on Peripheral Pulse Volume.

MICHAEL DEBAKEY, GEORGE E. BURCH AND ALTON OCHSNER.

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In view of clinical observations suggesting the possibility of ipsilateral arterial and arteriolar vasoconstriction in femoro-iliac thrombophlebitis,¹ the present investigations were undertaken to study some of the factors which may be concerned with such a phenomenon.

The influence of local chemical femoral thrombophlebitis upon the volume of pulsations was studied in the hind feet of 12 dogs. The volume of pulsations was determined plethysmographically and recorded continuously by Turner's² method, sensitive to volume

⁸ Almquist, H., and Stokstad, E., *J. Nut.*, 1937, **14**, 239.

¹ Ochsner, Alton, and DeBakey, Michael, *Surgery*, 1939, **5**, 491; also *J. A. M. A.*, in press.

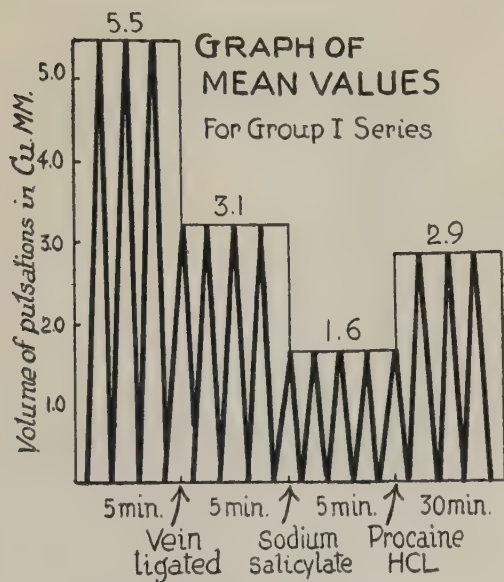
² Turner, R. H., *J. Clin. Invest.*, 1937, **16**, 777.

changes of 0.1 cu mm. By means of a specially constructed cellulose-acetate plethysmographic cup the apparatus was adapted to the hind foot of the dog. Ether anesthesia was used on all dogs during the period of observation and aseptic precautions were observed in the operative procedures. In all observations comparable venous segments were isolated and ligated with silk. The perivascular tissues were not disturbed except for a distance of a few mm at the sites of the proximal and distal ligatures. This venous segment included the femoral vein from just proximal to the saphenous entrance below to Poupart's ligament above. Venous pressures were determined directly³ in the saphenous vein at heart level before and after the venous ligations in most of the dogs. All observations were conducted under controlled atmospheric conditions, temperature 75° and humidity 50%.

The studies were divided into 5 groups of experiments as follows: (1) in 6 dogs (Nos. 1 to 6 inclusive) the venous segment in the left leg was exposed and ligated. Five minutes later 1 cc of blood was aspirated from the ligated segment and replaced by an equal quantity of 40% aqueous solution of sodium salicylate. Following another 5 minute interval the perivascular tissues of the entire venous segment were carefully infiltrated with 5 to 7 cc (depending upon the size of the dog) of 1% procaine hydrochloride. Observations were then continued for a period of 30 minutes. (2) In 2 dogs (Nos. 7 and 8) the Group 1 experiment was repeated on the left leg except that the procaine hydrochloride infiltration was done immediately after exposure of the vein and then followed by ligation and injection of the salicylate solution. (3) In 5 of the above dogs (Nos. 1, 2, 3, 6, and 8) 24 hours after resection of the right lumbar sympathetic ganglia and intervening chain, the respective procedures outlined in the first group of studies were repeated on the right leg. (4) On the left leg of dogs (Nos. 9, 10, and 11) the respective procedures outlined in the first group of studies were repeated except that the salicylate solution was injected into the perivascular sheath of the venous segment of the left leg. (5) In 3 dogs (Nos. 9, 10, and 12) 24 hours after resection of the right lumbar sympathetic ganglia and intervening chain, the procedures outlined in Group 4 were repeated on the right leg except that the perivascular tissues were not infiltrated with procaine hydrochloride.

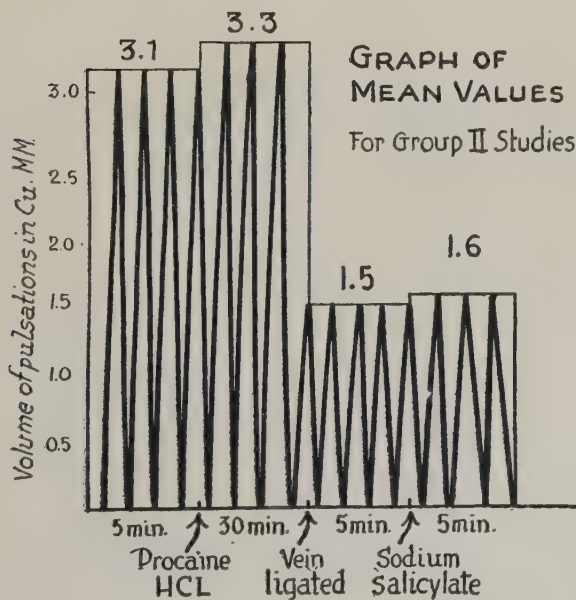
Whereas the sequence of each procedure in the 5 groups of ex-

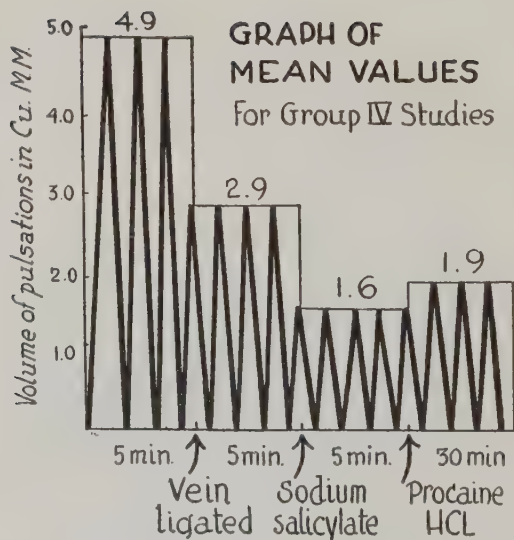
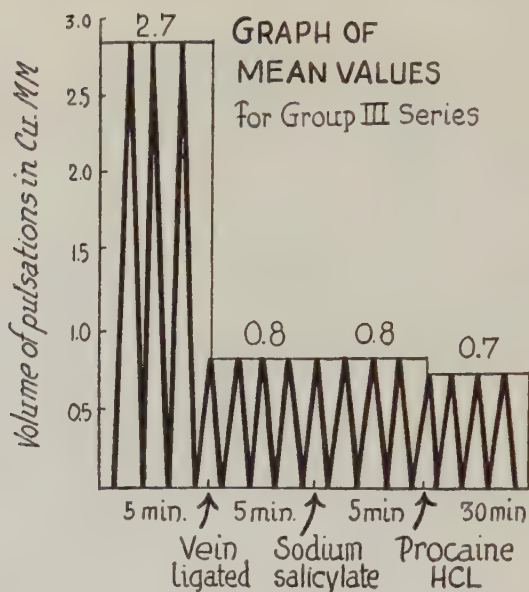
³ Burch, G. E., and Sodeman, W. A., *J. Clin. Invest.*, 1939, **18**, 31.

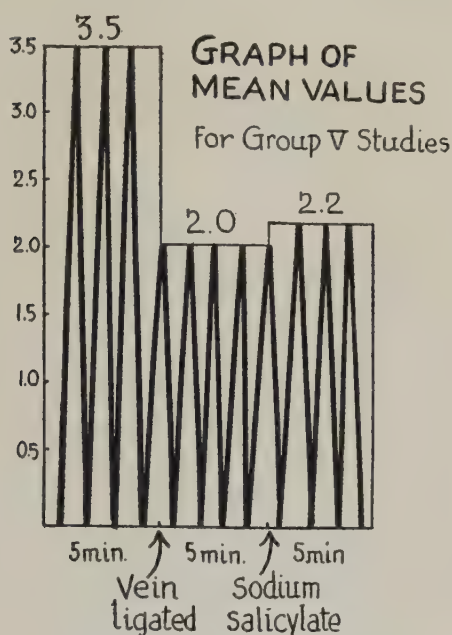


FIGS. 1 to 5.

Graphic representation of mean values of results obtained in the 5 groups of studies.







periments varied, the time periods of observation were the same for comparable procedures in all experiments.

Results. The mean results for each group of studies are illustrated in Figs. 1-5. Every dog in each of the five groups reacted in exactly the same manner as indicated by the corresponding graphs of the mean values, the individual variations being only one of degree. It was invariably found in all groups of studies that the volume of pulsations in the foot decreased markedly (52.5%) following ligation of the femoral vein reaching a maximum within 5 minutes. The decrease in volume occurred whether the lumbar sympathetic ganglia and intervening chains were intact or not. In the Group 1 studies, the volume of pulsations was decreased markedly (51.6%) following the introduction of the solution of sodium salicylate into the venous segment. This effect was abolished by the perivascular infiltration with procaine hydrochloride (Fig. 1). In the second group of studies in which the perivascular tissues were infiltrated with procaine hydrochloride before ligating the venous segment, the instillation of sodium salicylate into the venous segment did not affect the volume of pulsations in the foot (Fig. 2). The same results as obtained in Group 2 studies were obtained in Group 3 in which the lumbar sympathetic ganglia and chain were resected 24 hours before the observations were made (Fig. 3). In

the Groups 4 and 5 studies, similar to Groups 1 and 3 except that the chemical irritant was injected into the perivascular sheath of the vein, the results were similar to those obtained in Groups 1 and 3 respectively, in which the chemical irritant was placed intravenously (Figs. 4 and 5). The volume of pulsations decreased 55.2% following the perivascular chemical irritation in the fourth group of studies, a decrease quite similar to that following intravenous irritation. In this group of studies following the procaine hydrochloride infiltration, the volume of pulsations did not return to the values obtained after venous ligation, as in Group 1. This is probably due to the fact that the procaine hydrochloride solution did not permeate adequately the sclerosed perivascular tissues. Follow-up studies of the dogs revealed that volume pulsations returned to normal within a period varying from 3 days to 8 weeks.

Discussion. It is readily evident from all graphs that ligation of the main vein of the posterior extremity of the dog resulted in a marked diminution (52.5%) in the volume of pulsations in the foot. It was also observed that the venous pressure increased from a mean value of 7.7 cm H₂O before ligation to 97.0 after ligation. This effect was not influenced by the presence or absence of the sympathetic ganglia and intervening chain. At present, the explanation for this phenomenon can only be conjectured. However, investigations are being continued in an attempt to evaluate the factors concerned with its mechanism.

It is evident that a chemical irritant placed either in the lumen of the main vein of the extremity or in the perivascular tissues of this vein produces a marked diminution in the volume (51.6%) of peripheral pulsations. However interruption of nerve pathways by local infiltration with procaine hydrochloride at the site of the chemical irritation or by resection of the lumbar sympathetic ganglia and chain abolished this effect. This would suggest, therefore, that the decrease in volume pulsations following chemical phlebitis and periphlebitis is due to vasoconstrictor impulses initiated locally by the chemical irritant and coursing through the sympathetic ganglia in order to reach the terminal arterial vessels of the extremity.

Further Observations on the Red Pigments of Pellagra Urines.

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In an earlier study¹ it was noted that the amount of urinary coproporphyrin in 4 cases of alcoholic pellagra was not correlated with the Beckh-Ellinger-Spies test.² The latter investigators had found this test positive in many cases of pellagra and they believed that it indicated the presence of porphyrinuria. The test was employed by them in a quantitative as well as qualitative manner, and, unfortunately, has been recommended by others³ as a quantitative procedure for urinary porphyrin. In recent reports Spies^{4, 5} has stated that the test revealed the presence of porphyrin or porphyrin-like substances. In the writer's previous communication red pigments were described, differing markedly from porphyrins, particularly in their ready solubility in toluene and the difficulty with which they were extracted from organic solvents by hydrochloric acid. The Beckh-Ellinger-Spies (B.E.S.) test, however, was not correlated with the presence of red pigment in the toluene preservative. Subsequent study of the urines of additional cases of pellagra has shown that one or both of 2 red pigments may be present in varying amount. The first of these occurs as a chromogen and changes into a pink or red pigment upon the addition of hydrochloric acid to the urine. Particular attention has been given recently to the study of this chromogen in the urines of 2 cases of endemic pellagra. Two 24-hour samples were available from each of these cases.* The urine samples were sent to the writer from the

¹ Watson, C. J., *Proc. Soc. Exp. Biol. and Med.*, 1938, **39**, 514.

² Beckh, W., Ellinger, P., and Spies, T. D., *Quart. J. Med.*, 1937, **30**, 305.

³ Bray, W. E., *Synopsis of Clinical Laboratory Methods*, C. V. Mosby Company, St. Louis, 1938, p. 47.

⁴ Spies, T. D., Aring, C. O., Gelperin, J., and Bean, W. B., *Am. J. Med. Sc.*, 1938, **196**, 461.

⁵ Vilter, R. W., Vilter, S. P., and Spies, T. D., *Am. J. Med. Sc.*, 1939, **197**, 322.

* A small portion was removed from each sample, for the purpose of porphyrin and flavin determinations, in Birmingham. According to the note received with the urine specimens these patients were complaining of sore tongue, cracked lips, and abdominal distress of a burning type. They were evidently classified as pellagra cases at the time of shipment of the urines. In a subsequent communication, however, Dr. Spies stated his belief that both patients were suffering from riboflavin deficiency. They had not received nicotinic acid since last year at which time they were treated for pellagra.

Hillman Hospital in Birmingham, Alabama, through the courtesy of Dr. Spies. It had been noted in Birmingham that the B.E.S. test was positive in each urine. This was likewise true in Minneapolis. The substance responsible for the red color in this test, however, was not porphyrin in any of the 4 urines. The amount of coproporphyrin in each 24-hour sample was estimated fluorimetrically with the stufenphotometer. The values were unusually low: Case 1, 1st 24 hours: trace, too small to estimate; second 24 hours, 17.8 γ ; Case 2, 1st 24 hours: trace, too small to estimate; 2nd 24 hours, 14.1 γ . It should be emphasized again that all of the porphyrin (as determined by red fluorescence in ultraviolet light), was extracted by 5% HCl from the primary ether extract of the urine. The subsequent 25% HCl, containing the red pigment responsible for the positive B.E.S. tests, failed to show any trace of red fluorescence. Further study has revealed that this red pigment exhibits the various characteristics of uroosein, first described by Nencki and Sieber.⁶ The chromogen of this substance was identified by Herter⁷ as indolacetic acid. Nencki and Sieber had noted that this chromogen changes rapidly to uroosein if concentrated hydrochloric or sulphuric acid is added to the urine. The substance can then be concentrated readily in amyl alcohol by shaking with a small amount of this solvent. The alcohol assumes a rose red color and shows a fairly well defined absorption band at 552-559 m μ (max. 555-7). It should be noted that the spectroscopic absorption of uroosein in 25% HCl, as obtained in the B.E.S. test, is characterized by 2 weak bands: I 544, II 511.[†] The first of these is somewhat more intense, but both are relatively weak, broad and diffuse. These characteristics were noted in each of 4 urine samples received from the Birmingham cases. It was further noted that the chromogen, after extraction from the urine with ether and subsequent removal of the ether by evaporation on the water bath, gave the Salkowski reaction for indolacetic acid, as stressed by Herter.⁷

After preliminary extraction of the urine with ether, as in the B.E.S. test, the Nencki-Sieber test for uroosein on the extracted

⁶ Nencki, M., and Sieber, M., *J. f. Prakt. Chem.* (N.F.), 1882, **26**, 333.

⁷ Herter, C. A., *J. Biol. Chem.*, 1908, **4**, 253.

[†] Crystalline indolacetic acid has been found to exhibit the same color reactions with identical absorption spectra. This is true, however, only if a few drops of a dilute potassium nitrite solution have been added to the aqueous (or urine) solution prior to carrying out the B.E.S. or Nencki-Sieber tests. This supports Herter's opinion⁷ that the presence of nitrite in the urine is important to the development of the uroosein reaction.

urine, was negative. If the Nencki-Sieber test was first carried out, and the rose colored amyl alcohol, showing absorption at 556 m μ , was then separated, mixed with several volumes of ether and shaken with a saturated aqueous solution of sodium acetate, the pink color disappeared entirely. It was quickly regenerated by shaking with 2-3 cc of 25% HCl, as in the B.E.S. test.

Although the Nencki-Sieber test was not carried out in urine samples obtained from earlier cases of pellagra,¹ the entirely similar behaviour of the red substance in the 25% HCl of the B.E.S. tests leaves little doubt that it was the same which has been described above.

In none of the urines from the Birmingham cases has the toluene preservative so far become red or pink (toluene was added to a portion of each sample after arrival in Minneapolis). This is further indication that the red pigment previously noted in toluene preservatives differs from the substance giving the B.E.S. test. Some reference was made to this difference in the writer's earlier communication.¹ The possibility was considered then that the pigment going into toluene was a derivative of the B.E.S. pigment, although no direct evidence was obtained in favor of this concept. The similarity of the toluene soluble pigment to indirubin, or indigo red, was mentioned in the earlier report.¹ Further study has strengthened the belief that this pigment is indirubin, or a very closely related substance. It has now been observed in the urines of 7 additional cases, 3 of whom were pellagrins. One case of mild pellagra was, in fact, first recognized after the red toluene preservative had been noted. This patient was on the urological service receiving treatment for prostatic hypertrophy with pyonephrosis. He was 79 years of age and was found to be suffering as well from chronic fibroid pulmonary tuberculosis. The urine in this case was one of a number collected from various patients in a search for red pigments extracted by the toluene preservative. Urine samples from 30 patients have been observed in this respect for a considerable period of time. Red pigment in the toluene has been noted after from 2-10 days in 5 of these; 2 of these had extensive pulmonary and intestinal tuberculosis, one had Pott's disease with a large paravertebral abscess, and one had carcinoma of the prostate, suprapubic cystotomy and infected urine. The diagnosis in the fifth case is still obscure; this individual, a man of 60, was malnourished and had been on a deficient diet for over a year; hypochromic anemia, a mild hemorrhagic tendency, mild diarrhea and vomiting were present.

No organic lesion has been discovered. The urine in this case has repeatedly exhibited the red pigment in toluene; most interesting was the observation that the fresh urine at times gave strong B.E.S. and Nencki-Sieber tests for urorosein and simultaneously with the disappearance of these reactions the toluene gradually became pinkish red. A similar sequence of events was reported previously for a pellagra urine obtained from Baltimore.¹ In the present study the red pigment was also observed in the toluene preservative of urines from 2 cases of endemic pellagra.[‡] In one of these the amount was relatively large permitting isolation of the substance in crystalline form. The characteristics of the pigment extracted by toluene in all of these cases corresponded closely with those described by Rosin in an extensive study of indirubin or indigo red.⁸ As noted previously the substance crystallizes in the form of long narrow prisms of dark crimson color. The crystals sublime above 300°C in accordance with Rosin's description of indirubin. The lower sublimation temperature noted previously¹ may have been related to impurity. In contradistinction to urorosein, this substance goes into ether with a red or pink color, and cannot be removed in appreciable amounts by 25% HCl or 10% sodium hydroxide. The spectroscopic absorption is much less characteristic than in the case of urorosein; broad, diffuse, but relatively weak absorption is noted in the yellow and green, maximum from 530 to 550 mμ. In the Nencki-Sieber test, indirubin goes into the amyl alcohol but colors it a darker red, and the characteristic urorosein absorption band at 556 is not seen. Reduction to "white" indirubin by means of glucose and gentle heating, in an alcoholic solution, is readily effected; if the test tube containing this solution is shaken vigorously the upper portion coming in contact with the air again assumes a red violet color which fades when the shaking is discontinued ("Küpenreaktion").

The question of relationship of the 2 red pigments to one another cannot be answered at the present time. Assuming that they are urorosein and indirubin, as indicated in the foregoing, it is probable that a close relationship exists. This is suggested by their common origin from tryptophane, their chemical structure,⁹ and the repeated observation that as the urorosein reaction disappears the indirubin

‡ The writer is indebted to Drs. J. H. Musser and Alden Graves of the Charity Hospital, New Orleans, for their cooperation in arranging for the transmission of these urines to Minneapolis.

⁸ Rosin, H., *Virch. Arch.*, 1891, **123**, 519.

⁹ Dalmer, O., in Oppenheimer's *Hand. d. Biochem. des Menschen u. d. Tiere*. II. Aufl. Bd. I, pp. 257 and 261, 1924, G. Fischer, Jena.

color appears in the toluene preservative. Further investigation of this question and also of the question of relationship of vitamin deficiency to the appearance of these substances in the urine, is in progress. It seems noteworthy that both urochrome and indirubin have been encountered in a variety of diseases such as cancer, tuberculosis, and diabetes,^{6, 7, 8} all of which, however, are frequently associated with various deficiency states. Nencki and Sieber⁹ first encountered urochrome in the urine of a diabetic patient; it is therefore of interest that Spies and his associates⁵ have recently suggested correlation between cozymase deficiency in diabetic patients, and the presence of a positive B.E.S. test.

Conclusions. 1. The color noted in positive Beckh-Ellinger-Spies tests is not due to porphyrin. While a marked increase in urinary porphyrin, such as occurs in porphyria, would be productive of color, the color reaction as observed in pellagra and other diseases, is due to urochrome, first described by Nencki and Sieber. There is no evidence that the color reaction is due to any bile pigment derivative. 2. The urines of pellagra patients may contain either the chromogen of urochrome, or a red pigment extracted by the toluene preservative. It appears highly probable that this pigment is indirubin, although exact identification has not yet been made. 3. Both of the red substances may be noted in the urines of patients not having clinical pellagra. Further investigation is necessary to decide whether their appearance is related to deficiency of nicotinic acid or other essential substances.

10756 P

Metabolism of Two Di-deuterobutyric Acids as Indicated by Deuterium Content of Excreted Beta-Hydroxybutyric Acid.

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In order to obtain direct information as to what portions of the fatty acid molecule are convertible to the acetone bodies, a study has been made of the metabolism of the di-deuterobutyric acids. In the first series of tests alpha-beta and beta-gamma deuterobutyric

* Aided by a grant of the Rockefeller Foundation.

acids were fed in doses of 50 mg (as acetone) per 100 sq cm to male rats having an endogenous ketonuria as a result of a previous high-fat diet. The betahydroxybutyric acid separated by extraction of the urine was analyzed for deuterium content by the pressure float method of Rittenberg and Schoenheimer.¹ In 3 tests on alpha-beta deuterobutyric acid only approximately 4% of the extra betahydroxybutyric acid contained deuterium, while in a similar number of tests with the beta-gamma acid 17 to 25% of the betahydroxybutyric acid retained the deuterium. Control rats which received an equivalent concentration of deuterium oxide, excreted no deuterobetahydroxybutyric acid.

In the second series of tests beta-gamma deuterobutyric acid was fed to fasting female rats, which did not have an endogenous ketonuria, in amounts of 150 mg (as acetone) per 100 sq cm per day. In order to prove that the betahydroxybutyrate separated from the urine was not contaminated with sufficient deuterobutyrate (unmetabolized) to account for the deuterium found in the samples of the first series, the hydroxy acid was further purified by precipitation as the silver salt. In 9 different tests an average of 23% of the betahydroxybutyrate was found to contain deuterium. The purity of the silver salt was sufficiently high so that the deuterium could not have been present as a component of unmetabolized deuterobutyrate.

It is concluded that deuterium is retained to a considerable extent in betahydroxybutyrate when present on the gamma carbon (as occurs after feeding beta-gamma di-deuterobutyric acid) although it is almost completely lost when present on the alpha carbon (after administering alpha-beta deuterobutyrate). This procedure may be used for identification of the source of the acetone bodies. It also proves that ingested butyric acid is the source of urinary betahydroxybutyrate.

¹ Rittenberg, D., and Schoenheimer, R., *J. Biol. Chem.*, 1935, **111**, 169.

10757 P

Phasic Blood Flow in Coronary Arteries Obtained by a New Differential Manometric Method.

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An optically recording flow meter has been devised which accurately registers the phasic blood flow in the various vessels of the heart.

The rate of flow is measured by directing the blood through a thin orifice plate (or Pitot tube arrangement). The momentary acceleration of the blood so produced causes a lowering of the lateral pressure (for a short distance downstream) which is proportional to the square of the acceleration. The moment to moment differences between the lateral pressures above and below the orifice, recorded by a differential manometer, give the phasic changes in the rate of blood flow.

The upstream lateral pressure is led to a manometer tip with a 4 mm opening covered with a special rubber membrane 0.003 to 0.006 inch thick stretched 3 to 5 times. A waterproofed plano mirror (chip from a Bureau of Standards certified counting chamber cover slip) is mounted on the membrane by a peg or splint with special cement (to prevent hysteresis). The downstream lateral pressure is led directly to a water tight chamber (made of transparent Lucite) which surrounds the membrane. The light beam enters this chamber through a low diopter plano-convex lens (0.5 to 0.7). This lens should be at least 1.5 mm thick to prevent distortion under pressure. A small angle prism, capable of rotation through 360 degrees, mounted in front of the lens, corrects the prismatic effect of the Locke's solution with which the chamber and unit are filled. The whole assembly is mounted in the carriage of a Gregg manometer.

When filled with Locke's solution by means of a flexible tube the meter, as used in small vessels such as the coronary arteries, has a frequency of 80 to 120 double vibrations per second and for a differential pressure of 10 mm Hg gives a deflection of 40 to 60 mm at 4 meters camera projection distance. This sensitivity, which can be easily increased or decreased either by changing the orifice plate or the meter tip during the experiment, is sufficient to detect

flows as small as 10 cc per minute. At flows of 60 to 80 cc per minute the net loss of head in the stream is not more than 3 to 4 mm Hg. The meter has no appreciable lag (less than 0.001 second) and faithfully follows the sudden starting and stopping of flow produced by rotation of a stopcock. It gives correct flow figures for when alternating flow is produced through the meter by a reciprocating plunger the flow calculated from the flow meter curves varies from 2 to occasionally as high as 10% from the flows directly and simultaneously measured in a graduate.

The meter has been used to measure the phasic coronary flow in the *ramus descendens* of the dog under a variety of circulatory conditions. The loss of pressure head in the blood stream approximates 4 mm Hg. This is, however, insufficient to affect the vascular bed since continuous use of the meter over long periods of time causes no alteration of the blood flow. With good dynamic conditions prevailing the meter records show that the systolic coronary flow may be as large as that during diastole (for an equivalent time interval). The systolic and diastolic flows are separated by abrupt flow reductions during the isometric contraction period and late systole.

Such flows have been compared with the flow curves reconstructed from differential pressure curves taken at essentially the same time.¹ Such comparison of the flows obtained by the two methods confirms the conclusions drawn from constant pressure flow meter studies² that the differential pressure curves faithfully record the proper time relations and directional changes in flow but may underestimate the phasic changes in velocity. In addition the isometric retardation and the inflow during rapid ejection and isometric relaxation may be relatively greater than indicated by the differential pressures.

During elevation of systemic pressure (compression of the aorta or blood transfusion) or temporary reduction of the coronary blood supply (the flow being measured just after restoration of the normal blood supply) the flows increase markedly during both systole and diastole. Such flow increases are generally greater than indicated by the differential pressures. The reasons for these differences in coronary flow as measured by the two methods are now being studied.

¹ Green, H. D., Gregg, D. E., and Wiggers, C. J., *Am. J. Physiol.*, 1935, **112**, 362.

² Green, H. D., and Gregg, D. E., unpublished experiments.

10758

Survival of Litters from Adrenalectomized Rats Treated with Cortico-Adrenal Substitutes.

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Rats adrenalectomized several days prior to parturition will deliver normal litters but will not lactate sufficiently to raise them, if untreated, unless accessory adrenal cortical tissue is present.¹ Nelson and Gaunt² and Reece³ have shown the adrenal cortical hormone to be a necessary factor in the initiation of lactation.

Preparations of the corpus luteum hormone^{4, 5, 6, 7, 8} will prolong the survival period of young adrenalectomized rats. It has also been reported that adrenalectomized rats with ovaries heavily luteinized by gonadotropic hormones^{7, 9} survive longer than untreated adrenalectomized controls. NaCl and other salts will help support life and lactation in adrenalectomized animals. The salt therapy is enhanced by injections of adrenal cortical hormone, while the cortical hormone alone in adequate dosage can maintain life and lactation in adrenalectomized rats.¹⁰ The effect of testosterone propionate, directly or indirectly, on the female reproductive system^{11, 12} suggested its use as a cortico-adrenal substitute.

Pregnant albino rats were bilaterally adrenalectomized on the 17th to 21st day of gestation. After delivery control and experi-

¹ Gaunt, R., *Am. J. Physiol.*, 1933, **103**, 494.

² Nelson, W. O., and Gaunt, R., *PROC. SOC. EXP. BIOL. AND MED.*, 1937, **36**, 136.

³ Reece, R. P., *PROC. SOC. EXP. BIOL. AND MED.*, 1939, **40**, 25.

⁴ Gaunt, R., and Hays, H. W., *Am. J. Physiol.*, 1938, **124**, 767.

⁵ Gaunt, R., Nelson, W. O., and Loomis, E., *PROC. SOC. EXP. BIOL. AND MED.*, 1938, **39**, 319.

⁶ Greene, R. R., Wells, J. A., and Ivy, A. C., *PROC. SOC. EXP. BIOL. AND MED.*, 1939, **40**, 83.

⁷ Fischer, A., and Engel, M., *Rev. Franc. d'Endocrinol.*, 1938, **16**, 400.

⁸ Schwabe, E. L., and Emery, F. E., *PROC. SOC. EXP. BIOL. AND MED.*, 1939, **40**, 383.

⁹ D'Amour, M. C., and D'Amour, F. E., *PROC. SOC. EXP. BIOL. AND MED.*, 1939, **40**, 417.

¹⁰ Gaunt, R., and Tobin, C. E., *Am. J. Physiol.*, 1936, **115**, 588.

¹¹ Starkey, W. F., and Leathem, J. H., *PROC. SOC. EXP. BIOL. AND MED.*, 1938, **39**, 218.

¹² Nathanson, I. T., Franseen, C. C., and Sweeney, A. B., Jr., *PROC. SOC. EXP. BIOL. AND MED.*, 1938, **39**, 385.

mental litters were reduced to 5 young each to make their comparative growth records uniform. Since all the adrenalectomized females showed maternal care and attempted to suckle their litters, the day of death of the last rat in each litter was considered the time of complete lactation failure.

I. *Normal lactation.* No cases of lactation failure have been noted in this colony. As a standard for normal lactation, daily weight records were made on the litters of 5 unoperated, untreated mothers for 25 days. The average weight per young was 40.2 g on the 25th day.

II. *Ovariectomized—untreated.* To determine if the luteinization of the ovaries after delivery is necessary for normal lactation, 5 females were bilaterally ovariectomized on the day of delivery. One suckling rat was lost from each of 4 of these litters during the 25 day period. The average weight of the young surviving to the 25th day was 40.8 g. The loss of young from some of these litters indicates a slight disturbance in maternal care or lactation. The litters which survived to the 25th day were within the normal weight range of the controls.

III. *Adrenalectomized—untreated.* Since the survival¹³ and lactation of untreated adrenalectomized rats varies in different colonies, ten females were adrenalectomized as untreated controls. These animals survived an average of 13.6 (8-17 days). Three of the 10 absorbed their young. The average survival of the remaining 7 litters was 5.7 (3-8) days.

IV. *Adrenalectomized—1.05% or 2.0% NaCl drinking solutions.* Seven females which were given 1.05% or 2.0% NaCl solutions to drink, survived an average of 37.3 (8-77) days and their litters 6.5 (3-10) days. Three of these females died on the 37th, 67th and 77th day after adrenalectomy, 3, 4 and 12 days respectively after the mothers were given tap water to drink in place of the salt solutions. The short survival of these litters indicates that although the NaCl solutions did prolong the survival time of the mothers, this therapy is not a complete substitute for the adrenal cortex of lactating rats.

V. *Adrenalectomized—crystalline progesterone.** Five females survived an average of 17.8 (10-29) days and their litters 6.6 (2-11) days when given single daily injections of crystalline progesterone totalling 2 mg over 2 days; 7 mg over 3 days; 22 mg over

¹³ Gaunt, R., Gaunt, J. H., and Tobin, C. E., *PROC. SOC. EXP. BIOL. AND MED.*, 1935, **32**, 888.

6 days; 42 mg over 10 days; and 55 mg over 7 days. The apparent failure of lactation indicated by the early death of the litters was probably due to the androgen-like action of progesterone¹⁴ which would tend to inhibit lactation. The survival time of the females was increased and found to be proportional to the total progesterone dosage even though the injections were not continued throughout the whole survival period of the females but were stopped with the death of the litters.

VI. *Adrenalectomized—crude progestin*.* Five females survived an average of 7.8 (5-10) days and their litters 4.0 (2-5) days with daily injections of progestin totalling 2.4 mg over 4 days; 3.6 mg over 6 days; 10.8 mg over 5 days; 15.2 mg over 6 days; and 22.5 mg over 5 days. Injections were stopped with the death of the litters. The failure of this substitute therapy to support lactation and prolong the survival period of the females was probably due to the estrogenic material and other impurities present in this crude progestin preparation. It has been shown that estrogens are toxic to adrenalectomized animals⁴ and the inhibitory effect of estrogens on lactation is well established.

VII. *Adrenalectomized—Antuitrin S*.* Nine females were treated with Antuitrin S: one injected with 25 r.u. and one with 50 r.u. on the day before parturition; 4 animals, one injection on the day of parturition: 10 r.u., 20 r.u., 25 r.u., and 50 r.u.; 3 animals, one injection on the day of parturition followed by another injection 2 or 4 days later: 10 and 15 r.u., 25 and 25 r.u., and 50 and 50 r.u. Two of the females which were killed on the 51st day had accessory adrenal cortical tissue, but neither raised its litter beyond the 9th day. One was autopsied on the 8th day after injection, 4 days after her litter died. The other 6 females survived an average of 18.3 (13-30) days and their litters 6.4 (3-13) days. One female of this group which was injected the day before parturition had a survival period of 30 days and her litter, 13 days. The ovaries of these females were heavily luteinized at the time of death or autopsy.

IX. *Adrenalectomized—testosterone propionate*.* Five females survived an average of 18.4 (13-31) days and their litters 3.0 (1-5)

¹⁴ Greene, R. R., Burrill, M. W., and Ivy, A. C., *Endocrinol.*, 1929, **24**, 351.

* The author is indebted for the gifts of the following material which made this work possible: Crystalline progesterone and testosterone propionate, Dr. Erwin Schwenk, The Schering Corporation; Antuitrin S, Parke, Davis & Company; crude progestin, Dr. W. M. Allen, The University of Rochester, School of Medicine and Dentistry.

TABLE I.
Survival of Adrenalectomized Females and Their Litters After Cortico-adrenal Substitute Therapy.

Treatment	No. of animals	Avg survival Range of litters, days	Avg survival Range of mother, days
Adrenalect. untreated	10	5.7 (3-8)	13.6 (8-17)
1.05% and 2.0% NaCl sol.	7	6.5 (3-10)	37.3 (8-77)
Crystalline progesterone	5	6.6 (2-11)	17.8 (10-29)
Crude progestin	5	4.0 (2-5)	7.8 (5-10)
Antuitrin S	9	6.4 (3-13)	18.3 (13-30)
Testosterone propionate	5	3.0 (1-5)	18.4 (13-31)

days after daily injections of testosterone propionate (1-1.5 mg daily) totaling 11 mg over 11 days; 12.5 mg over 13 days; 14 mg over 13 days; 14 mg over 15 days; and 15 mg over 16 days. Injections were started one or two days before and continued 10 to 14 days after parturition. The failure of lactation after these injections is similar to that reported by Folley and Kon¹⁵ for normal rats after testosterone propionate injection. This experiment indicates that the dosage of testosterone propionate used can slightly prolong the survival of adrenalectomized mothers.

In other experiments^{5, 6, 8, 9} young adrenalectomized rats were used to test the effect of cortico-adrenal substitutes (their survival time being shorter and less variable than that of the older animals used in this experiment). The average length and range of survival of these adult adrenalectomized females (Table I) indicates that the dosage of some of these substitutes for the hormone of the adrenal cortex will prolong their survival time as compared with that of untreated adrenalectomized females. In none of these experiments was the survival time of the litters (Table I) enhanced by this therapy.

Conclusions. These experiments indicate that NaCl drinking solutions, crystalline progesterone, crude progestin, testosterone propionate, and Antuitrin S, in the dosages used as cortico-adrenal substitutes, will not enhance lactation as determined by the survival of the litters from adrenalectomized females. As compared with the survival of untreated adrenalectomized controls, crude progestin reduced the survival time of the females, whereas the other substitutes prolonged the survival time of the females.

¹⁵ Folley, S. J., and Kon, S. K., *Proc. Roy. Soc. London*, 1038, sB, **124**, 476.

10759

Relation of Nutrition to Gastric Function. I. An Experimental Method.

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We have developed an experimental procedure for studying the relation of nutrition to gastric function with the rat as the subject. Tentatively, we are determining the total acidity, the free HCl, and the mucin of gastric juice as a measure of gastric function. By "mucin" we mean that material which is precipitated by adding 4 volumes of alcohol to gastric juice and is determined as glucose by the orcinol method of Tilmans and Philippi.¹ Later we plan to study the enzyme and other constituents of the gastric juice.

As gastric stimulants we have used mecholyl (acetyl- β -methylcholine chloride) and histamine. The dosage used per kilo of body weight was 0.01 mg for mecholyl and 0.03 mg of ergamine phosphate for histamine. Each stimulant was dissolved in physiological saline and injected subcutaneously.

Our experimental procedure is as follows: Rats are placed in individual false-bottom cages and fasted for 48 or 72 hours. A meshed-wire jacket is placed upon the torso of each rat during the fasting period to prevent coprophagy and to keep the animal from licking its body, a process by which the rat ordinarily ingests considerable hair. This preparatory period of fasting in a jacket permits the rat's stomach to be cleared of solid contents and makes possible the collection of uncontaminated juice.

When a test is made the animal is anesthetized with nembutal and during the experiment nembutal is injected in small doses as needed to maintain anesthesia. A small incision is made in the abdomen and a tight ligature is tied around the duodenum near the pyloric sphincter. Such a ligature does not interfere with the nerve supply nor appreciably with the blood supply to the stomach and permits a secretory response of the whole stomach. The abdomen is closed with clamps. The gastric stimulant (mecholyl* or histamine) is injected subcutaneously at 15-minute intervals. One-half hour after beginning stimulation the abdomen is opened and the contents of

¹ Tilmans, J., and Philippi, K., *Biochem. Z.*, 1929, **215**, 36.

* We are indebted to Merck and Company for generous contributions of Mecholyl.

the stomach are removed by inserting a 20 gauge needle through a point in the stomach wall in the region of the rumen and aspirating with a syringe. In the same manner the stomach is emptied of gastric juice again in one-half hour and thereafter at one-hour intervals for 2 or 3 hours. The volume of the juice in each collection is recorded and the juice is analyzed.

With this experimental procedure regurgitation of intestinal contents into the stomach cannot occur; also saliva is not swallowed, a fact we demonstrated by placing a cotton plug in the esophagus. With the probable exception of the first collection, which might contain regurgitated material, the samples of gastric juice obtained are not contaminated by extra-gastric fluids. The only possible contamination that may occur is from bleeding at the sites of the needle punctures. This is very slight, and we have satisfied ourselves by control studies that it does not alter the analyses for the constituents reported in this paper.

For analysis a sample of juice (0.5 to 0.1 cc) is pipetted into a 50 cc conical bottomed centrifuge tube, a drop of Topfer's reagent is added, and the free HCl is titrated with 0.05 N NaOH from a microburette. A drop of phenolphthalein is added and the total acidity is titrated. The juice is made slightly acid again by adding acetic acid, and 4 volumes of 95% alcohol are added. The tubes are allowed to stand over night to permit maximum precipitation of the mucin, after which the content of the latter is determined.

Results obtained with the technic as outlined above upon 12 rats of weights ranging from 136 to 263 g, 6 receiving mecholyl stimulation and 6 histamine stimulation, are shown in Fig. 1. The rats used were young adults. These results are in agreement with the data obtained upon a larger number of normal rats used in developing this procedure. For total acidity the broken line curves represent individual values and the solid line is the average curve. The solid line curves for mucin and volume are composite curves showing the average values for the 6 rats in each case.

The highest average total acidities observed with mecholyl and histamine are 84 and 96 cc of 0.1 N acid per 100 cc of juice, respectively. The response is more rapid following histamine. With histamine the peak of the curve is observed most often in the half-hour collection, while with mecholyl the peak most often occurs between the first and second hour collections. The free HCl values obtained in these studies in general parallel the total acidity data, being 60 to 70% of the latter. The average values per 100 cc of gastric juice for mucin range from 23 to 40 mg as glucose following mecholyl stimulation, and 16 to 51 mg after histamine. The volume curve

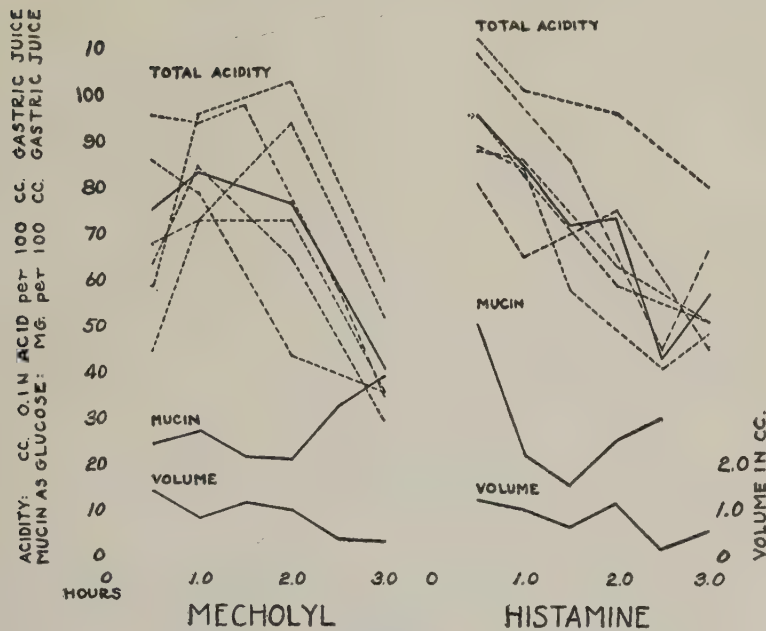


FIG. 1.

Effect of mecholyl and histamine upon gastric secretion in the rat.

shows a close parallelism between the concentration of acid and the amount of juice secreted.

The first sample of juice collected in each experiment may have been contaminated by residual material, hence the first point on all of the curves in Fig. 1 is probably atypical.

In general the degree of acidity, the amount of mucin, and the volume secreted are about the same with mecholyl stimulation as with histamine stimulation.

From the studies we have made thus far it appears that there is considerable similarity in the gastric secretions of the rat and man. With 10 human subjects, Helmer² found an average total acidity ranging from 82 to 111 cc of 0.1 N acid per 100 cc of gastric juice in a study by the fractional method following the administration of 0.5 mg of histamine hydrochloride. Our average curve with 6 rats receiving histamine ranges from 96 to 44 cc of 0.1 N acid per 100 cc of gastric juice. For mucin Helmer obtained values ranging from 12 to 18 mg per 100 cc as glucose. Our average curve for mucin with 6 rats after histamine stimulation shows values ranging from 16 to 51 mg per 100 cc as glucose. Our data with rats are

² Helmer, O. M., *Am. J. Physiol.*, 1934, **110**, 28.

thus comparable to the findings of Helmer for human subjects after the administration of a single dose of histamine.

The data of this report thus seem to justify the assumption that the rat is a subject with which studies of gastric function can be made that may have a bearing upon the mechanism of gastric secretion in man.

10760 P

Size of the Extracellular Compartment of Skeletal Muscle.

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The water of the animal body may be roughly divided into that contained within the cells, the intercellular fluid, and that contained in the fluid which bathes the cells but is not enclosed by the cell membrane, the extracellular fluid. There have been numerous attempts in recent years to determine the relative size of each of these compartments. In general, the methods employed consist of either (1) the determination of the amount of dilution of an injected, non-toxic foreign substance which does not enter the cell but is distributed uniformly throughout the extracellular water and (2) by the measurement of the amount of some normal constituent which is limited to and uniformly distributed throughout the extracellular water. Sodium thiocyanate has been suggested as a suitable substance for the first method¹⁻⁴ and chloride determination most convenient for the second method.⁵⁻⁹

¹ Crandall, L. A., and Anderson, M. X., *Am. J. Dig. Dis. Nutr.*, 1934, **1**, 126.

² Laviates, P. H., Bourdillon, J., and Klinghoffer, K. A., *J. Clin. Invest.*, 1936, **15**, 261.

³ Brodie, B. B., and Friedman, Max M., *J. Biol. Chem.*, 1937, **120**, 511.

⁴ Wallace, S. B., and Brodie, B. B., *J. Pharm. and Exp. Ther.*, 1937, **61**, 397, 412.

⁵ Wallace, S. B., and Brodie, B. B., *J. Pharm. and Exp. Ther.*, 1939, **65**, 214.

⁶ Amberson, William R., Nash, Thomas P., Mulder, Arthur G., and Binns, Dorothy, *Am. J. Physiol.*, 1938, **122**, 224.

⁷ Hastings, A. B., and Eichelberger, L., *J. Biol. Chem.*, 1937, **117**, 73.

⁸ Peters, John P., *Body Water*, p. 133, 1935, Charles C. Thomas, Baltimore, Md.

⁹ Harrison, H. E., Darrow, D. C., and Yannet, H., *J. Biol. Chem.*, 1936, **113**, 515.

Following the intravenous injection of sodium thiocyanate in the cat we find that this substance distributes itself through about 25 to 40% of the body weight. In 2 experiments on anesthetized cats sodium thiocyanate (148 mg and 177 mg per kilo body weight) was injected intravenously, 60 minutes allowed for its dilution, carotid blood taken for sodium thiocyanate and chloride analyses and the animals then sacrificed by asphyxia. Each animal was dissolved in normal KOH, digested in nitric acid and an aliquot taken for chloride analysis. Assuming the total chloride content of the animal body, with the exception of the red blood cell, is in the extracellular compartment and is in equilibrium with the blood plasma, we find that the thiocyanate and chloride available volumes are quite similar (Table I).

TABLE I.
Volume of Extracellular Fluid in the Cat.

Exp. No.	Wt in kg	NaCNS available Vol./kg body wt	Chloride available Vol./kg body wt
1	1.98	288 ml	316 ml
2	1.65	288 "	320 "

The thiocyanate and chloride available volume of muscle was determined as follows: sodium thiocyanate, 124 to 155 mg per kg body weight as a 5% solution in distilled water, was injected intravenously into cats anesthetized with dial (60 mg per kg body weight intraperitoneally). Sixty minutes were allowed for the distribution of thiocyanate throughout its available fluid after which time 10 ml of blood were taken from the carotid artery for thiocyanate¹ and chloride¹⁰ analyses. The animal was then killed by asphyxia. The various muscles taken for analysis were dissected out, carefully weighed, dried to constant weight in an oven kept at 105°C, dissolved in normal KOH and an aliquot of this taken for thiocyanate³ and for chloride¹⁰ analysis.

From these determinations the thiocyanate and chloride available water per 100 g of muscle is obtained as follows:

$$\text{Thiocyanate available volume} = \frac{\text{CNS in mM per 100 g of muscle}}{\text{CNS in mM per g of serum water}}$$

$$\text{Chloride available volume} = \frac{\text{Chloride in mM per 100 g muscle}}{\text{Chloride in mM per g serum water}}$$

The results from these determinations are shown in Table II.

¹⁰ Sunderman, F. W., and Williams, P. J., *J. Biol. Chem.*, 1933, **102**, 279.

TABLE II.
Thiocyanate and Chloride Available Volume of Skeletal Muscle of the Cat.

Organ	Experiment 12.		Avg Values			
	Chloride available Vol. in ml/100 g organ	CNS available Vol. in ml/100 g organ	Chloride Vol. % total	NaCNS Vol. % total	Chloride Vol. % total	CNS Vol. % total
Biceps f	11.5	12.3	15.0	15.8	13.6	17.2
Triceps b.	13.7	17.1	18.3	22.9	13.4	17.5
Gastrocnemius	11.9	13.9	15.6	18.4	16.2	16.2
Temporalis	16.0	21.0	20.9	27.4	16.8	22.5
Sternomastoideus	27.4	29.2	36.0	38.2	24.0	31.3
Trapezius	18.5	17.5	24.2	23.2	20.0	26.3
Diaphragm	24.1	21.2	31.4	27.6	29.3	38.0
Rectus a.	30.2	25.9	29.6	33.9	37.5	49.0
Heart	31.0	35.7	39.0	44.9	32.9	38.0
						41.5

The total thiocyanate and chloride available volumes of the normal cat are of similar magnitude. This distribution is not exclusively extracellular, inasmuch as CNS and chloride penetrate the red cell,¹¹ the digestive glands and are found in excessive quantities in the subcutaneous tissues of the skin (to be published elsewhere). In the muscles, chloride and thiocyanate are distributed through approximately the same volume of fluid. This volume differs from muscle to muscle and to some extent from animal to animal for any given muscle. It is not improbable that this distribution, at least in the thick muscles, is limited to the extracellular compartment.

10761

A Method for Preservation of Oxalated Plasma Clot for Fibrinolytic Tests.

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Oxalated plasma usually loses its ability to form a clot upon addition of calcium chloride when kept in the icebox for 72 hours.

We have been testing the fibrinolytic power of hemolytic streptococci immediately after isolation and at regular intervals thereafter to observe any changes in this property and its relation to other biological characteristics of the organism. A susceptible plasma clot from an individual was used repeatedly, necessitating a considerable number of bleedings, resulting usually in loss of time and material. For these reasons we decided to search for a method to preserve plasma while retaining its ability to clot readily and maintaining its susceptibility to lysis unaffected. This has been accomplished by the following method:

Human blood from a susceptible person was obtained and distributed in 5 cc amounts into small glass bottles, each containing 10 mg of potassium oxalate in the form of the dry powder. After mixing well by gentle shaking, the plasma was separated by centrifugation. The susceptibility of this plasma to the lytic action of hemolytic streptococci was tested, employing the original technic of

¹¹ Gregersen, M. I., and Stewart, J. D., *Am. J. Physiol.*, 1939, **125**, 142.

Tillett and Garner.¹* A 16- to 20-hour-old culture in neopeptone broth containing 0.02% glucose to which a drop of defibrinated rabbit blood was added at the time of inoculation, was used in all the tests. Transplants were always made to this medium from the stock cultures on blood agar. From the growth thus obtained a second transplant to a tube of glucose neopeptone blood broth was made. The growth from this second transplant was actually employed in the test. Clotting took place within 10 minutes. Dissolution was complete in from 10 to 15 minutes.

The fresh plasma was distributed in 0.3 cc amounts into special all glass containers of 5 cc capacity.[†] The material was then dehydrated by the Cryochem process using the degassing-self-freezing procedure described by Flosdorf and Mudd.² The material was processed for 22 hours, at the end of which the tubes were sealed under the original vacuum. The dehydrated material was kept in the icebox.

We have had some difficulty in obtaining the material in perfectly frozen condition with the degassing-self-freezing procedure. The plasma had the porous appearance described by other authors in some cases, but in other instances it looked slightly gelatinous. We feel that in localities where dry ice can be obtained, initial freezing with it will greatly simplify the procedure and improve the solubility of the final product.

To perform the fibrinolytic tests enough distilled water was added to the tubes of dehydrated material to restore the original volume. Solubility was accomplished readily by gentle shaking. Samples of the plasma in the dehydrated form were tested at monthly intervals for its ability to form a firm clot and for its susceptibility to lysis. Similar technic and reagents as employed for testing the fresh plasma were used in all tests. The last sample tested (kept for 8 months in the icebox) clotted in 10 minutes, and dissolution was complete in 18 minutes.

The ability of plasma to clot after addition of calcium chloride and its susceptibility to lysis remained constant after 8 months when the material was dehydrated by the above procedure.

* Strain Co. of hemolytic streptococcus kindly sent to us by Dr. Tillett was used.

¹ Tillett, W. S., and Garner, R. L., *J. Exp. Med.*, 1933, **58**, 485.

[†] Catalog No. 112—Cryochem Apparatus (Flosdorf-Mudd). F. J. Stokes Machine Co. of Philadelphia. A model No. 101 Cryochem apparatus was used.

² Flosdorf, E. W., and Mudd, S., *J. Immunol.*, 1938, **34**, 469.

10762

Schick Reaction and Menstrual Cycle.

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The possibilities of changes in resistance to infection at the time of menstruation have been widely discussed but there are few direct observations on the subject. Only in the case of the common cold,¹ herpes febrilis² and hemolytic streptococcus infections³ has it been shown, with any degree of certainty, that resistance may be lowered at this time. A number of attempts have been made to measure periodic fluctuations in the anti-infectious agencies of blood and tissues. Thus, Geller⁴ has demonstrated that the bactericidal titer of blood is depressed premenstrually, and Fisher⁵ reports a lowering in the disinfecting power of the skin at menstruation. In studying the virucidal properties of the blood at different times of the cycle, Jungeblut and Engle⁶ found that, although the ability to neutralize poliomyelitis virus changed, there was no uniform correlation with any given phase of the cycle. Ross⁷ reports the titer of natural protective substances against type II pneumococci as being higher during the first days of the menstrual cycle than during the latter part; and Dressel and Keller⁸ found that serum from menstruating women was bactericidal for the anthrax bacillus while serum from women who were not menstruating exhibited no such power. It is clear from the references quoted above that the effect of the menses on resistance to different diseases is not uniform and that each disease must be studied individually.

The physiological changes in capillary function that occur at the beginning of the menses are far from being understood; but Brewer⁹ has recently contributed data to suggest that capillary fragility may be greatly increased during the first 2 days of the menstrual period.

¹ Maciejewski, K., Thesis, Berlin, 1930.

² Lauda, E., and Luger, A., *Ergeb. inn. Med.*, 1926, **30**, 377.

³ Geller, F., and Sommer, W., *Arch. Gynækol.*, 1927, **131**, 293.

⁴ Geller, R., *Muench. Med. Woch.*, 1925, **72**, 1686.

⁵ Fisher, V., *PROC. SOC. EXP. BIOL. AND MED.*, 1931, **28**, 952.

⁶ Jungeblut, C. W., and Engle, E. T., *J. Immunol.*, 1933, **24**, 267.

⁷ Ross, V., and Peizer, L. R., *PROC. SOC. EXP. BIOL. AND MED.*, 1936, **35**, 432.

⁸ Dressel, E., and Keller, W., *Z. Hyg.*, 1922, **97**, 51.

⁹ Brewer, J. I., *Am. J. Obst. and Gynecol.*, 1938, **30**, 597.

Before, Benda¹⁰ had already noted that cell permeability is increased during the time of the flow. In view of the fact that one of the outstanding properties of diphtheria toxin is its ability to produce capillary damage,¹¹ it seemed of interest to investigate whether the Schick reaction could be used as an indicator for any possible changes in capillary resistance at different phases of the cycle.

Fluctuations in Schick-reactivity have previously been observed in connection with seasonal studies.¹² However, when Schick tests are repeated in the same individual over short periods of time—and specific exposure can be ruled out—a remarkable tendency for stability of the reaction becomes evident.¹³ The only exception to this rule, apparently, is the influence that fatigue¹⁴ and ultraviolet irradiation¹⁵ may exert in changing negative reactions temporarily to positive ones or making positive reactions more strongly positive.

Thirteen female medical students, aged from 20 to 25 years, were given repeated Schick tests. This number included 9 who were previously known to be positive reactors, and 4 who were Schick negative. These individuals were divided into 2 groups: The first group of 6 was given the initial Schick test at the beginning of the menses and the second test during the interval; in the other group of 7, the procedure was reversed. This arrangement was made so as to eliminate any possible error that might be introduced through partial immunization resulting from the initial antigenic stimulus. No evidence was found, however, that any such immunity developed since many of the second reactions were stronger than the first.

For the purpose of additional control, a group of 6 male students were Schick tested twice, at 14-day intervals. This was done in order to determine the uniformity of the test in repeated injections in the same individual, in the absence of any cyclic changes.

All injections were done intradermally on the flexor surface of the forearm, alternating arms being used for successive tests. One lot of toxin (N. Y. City Board of Health) was used throughout and all reactions were controlled by tests with heated toxin. Read-

¹⁰ Benda, R., *Muench. Med. Woch.*, 1925, **72**, 1686.

¹¹ Gautrelet, J., and Gautrelet, M., *Presse méd.*, 1935, **43**, 961.

¹² St. Tubiaz, M., *Office internat. d'hyg. publ.*, 1932, **24**, 2000; Nelis, P., *C. R. Soc. Biol.*, 1934, **115**, 1178.

¹³ Pintozzi, V., *C. Bakt. Ref.*, 1933-34, **112**, 59.

¹⁴ Zlatogoroff, S. I., and Kostereff, S. A., *C. R. Soc. Biol.*, 1931, **106**, 96; Feullie, P., Thiry, P., and Blancardi, C., *C. R. Soc. Biol.*, 1934, **115**, 367.

¹⁵ Bratusch-Marrain, A., and Asperger, H., *Med. Klin.*, 1932, **28**, 1310.

ings were taken at 24, 48 and 72 hours and the size of the area of inflammation was recorded as well as the degree of redness and edema. The last reading was used for final evaluation.

No significant changes were found in the male group. In the female group, however, the majority of the individuals showed considerable variation in intensity of the reaction. These changes involved the area and the degree of redness as well as the extent of edema; if one or more of these criteria was more pronounced whereas the others remained stable, the whole reaction was listed as more severe in our records. On the basis of such an interpretation it was found that of 9 Schick-positive individuals, 6 exhibited a severer reaction at the beginning of the menstrual period than during the interval while one reacted less strongly and 2 showed no change whatsoever. Among the 4 Schick-negative reactors, 3 remained negative on repeated injections; however, one individual who had given a completely negative reaction during the interval showed a distinct but mildly positive reaction at the beginning of the menses. Two individuals in the Schick-negative group gave pseudoreactions to the heated toxin. In one instance this pseudoreaction remained unchanged; in the other, a definite increase in severity was noted at the time she was menstruating.

The above data are of interest in demonstrating that susceptibility to a capillary poison, like diphtheria toxin, may be increased during the onset of menstruation.

10763

Sulfanilamide in Experimental Tuberculosis.*

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Rich and Follis¹ reported an inhibitory effect of sulfanilamide on experimental tuberculosis in guinea pigs. Doses of 200 and 500 mg were given daily, beginning 3 days before the animals were infected subcutaneously with human tubercle bacilli, and treatment

* This work was supported by a grant from the National Tuberculosis Association.

¹ Rich, A. R., and Follis, R. H., Jr., *Bull. Johns Hopkins Hosp.*, 1938, **77**, 621.

was maintained throughout the experiment. Similarly, Greey, Campbell and Culley,² using human bacilli, observed some inhibitory effect in guinea pigs, on a dosage of 300 mg of sulfanilamide; while Buttle and Parish³ obtained very favorable results with 250 mg administered *per os*. Ballon and Guernon,⁴ after experimenting with doses up to 680 mg, found that daily treatment with 340 to 380 mg of sulfanilamide was tolerated by guinea pigs, and had an inhibitory effect. However, Dietrich,⁵ using prontosis (100 mg per kilogram of body weight), observed no favorable influence of this drug on guinea pigs infected with human bacilli, although he treated his animals 14 to 16 days before infection. Smithburn⁶ used sulfanilamide in doses of 250 mg (50 mg less than Rich and Follis' most successful dosage) and obtained no beneficial results; while Kolmer *et al.*⁷ treated guinea pigs with 6 derivatives of sulfanilamide, in the form of sodium salts, using doses varying from 200 to 500 mg, and were equally unsuccessful. An early trial on a small number of animals suggested to us that sulfanilamide was not effective in experimental tuberculosis; but after learning of the results achieved by Rich and Follis,¹ we decided to repeat our experiment on a larger scale.

Experiment I. Fifty-four guinea pigs, averaging 365 g in weight, and negative to tuberculin, were infected subcutaneously in the groin with 0.0001 mg of virulent bovine tubercle bacilli (B1). Twelve animals remained untreated and served as controls. Forty-two animals were treated with sulfanilamide (Merck & Co.). The treated animals were divided into the following 3 groups: Group I: 14 guinea pigs received 100 mg sulfanilamide daily in 2 divided doses, 7 by oral administration and 7 by subcutaneous injection. Group II: 14 guinea pigs received 200 mg sulfanilamide daily in 2 divided doses, 7 *per os* and 7 by subcutaneous injection. Group III: 14 guinea pigs received 300 mg sulfanilamide daily in 3 divided doses, 7 *per os* and 7 by subcutaneous injection. At the end of the 5th, 6th, 7th and 8th week, animals from each group were sacrificed in consecutive series. In the first 3 series, one guinea pig from each group and 2 control animals were killed; the fourth

² Greey, P. H., Campbell, H. H., and Culley, A. W., *Proc. Soc. Exp. Biol. and Med.*, 1938, **39**, 22.

³ Buttle, G. A. H., and Parish, H. J., *Brit. Med. J.*, 1938, **4058**, 776.

⁴ Ballon, H. C., and Guernon, A., *J. Thor. Surg.*, 1938, **8**, 188.

⁵ Dietrich, H. F., *Am. Rev. Tuberc.*, 1938, **33**, 389.

⁶ Smithburn, K. C., *Proc. Soc. Exp. Biol. and Med.*, 1938, **38**, 574.

⁷ Kolmer, J. A., Raiziss, G. W., and Rule, A. M., *Proc. Soc. Exp. Biol. and Med.*, 1938, **39**, 581.

series included all the remaining guinea pigs. Macro- and microscopic readings were made from the lung, liver, spleen, and the mesenteric, superficial and deep inguinal lymph glands, in order to determine the extent of the lesions.

Results. Series 1: Eight guinea pigs were autopsied 35 days after infection. Macroscopic examination showed early tuberculosis in all the animals. The treated guinea pigs did not differ in any significant manner from the untreated. The regional glands were slightly enlarged, with a small degree of caseation evident in all but the control animals. In the guinea pigs which had received daily doses of 200 and 300 mg of sulfanilamide by injection, the livers were clear, but the spleen showed early tubercles. Lungs were congested, but no tubercles were found.

Series 2: The 8 guinea pigs of this series were autopsied 41 days after infection. Macroscopic examination revealed no significant variation between the animals treated with sulfanilamide and those left untreated. Lungs were clear in all but one control animal. Regional glands were much enlarged, with beginning caseation. Involvement of the livers and spleens varied considerably in individual animals, but this variation extended throughout the entire group.

Series 3: Autopsy readings were made on these 8 guinea pigs 48 days after infection. Again macroscopic examination revealed no significant variation among these animals. Lungs were all slightly involved; the regional lymph glands were very much enlarged and showed moderate caseation. The least involvement was observed in the animals from Group I. Spleens were enlarged and contained tubercles in every instance.

Series 4: The remaining guinea pigs were autopsied 54 days after infection. No significant difference in the extent or the degree of infection was seen between the treated animals and the untreated controls.

Microscopic examination of slides prepared from the lungs, livers, spleens, and lymph glands corresponded with the macroscopic findings.

Experiment II. Twelve guinea pigs were divided into 2 groups of 6 animals. Group I was treated for 3 days with sulfanilamide in 100 mg doses administered orally 3 times daily. These guinea pigs were then injected subcutaneously with 0.0001 mg bovine strain (B1) tubercle bacilli, and the sulfanilamide treatment continued throughout the course of the experiment. The animals in

Group II were infected but remained untreated for control purposes. One guinea pig from each group was autopsied during the 6th week after infection, and the rest of the animals during the 7th week.

Results. Set 1: Two guinea pigs were autopsied 37 days after infection. Macroscopic readings of the necropsy findings were similar in the 2 animals, although the tuberculous lesions of the untreated guinea pig were perceptibly more advanced than those of the sulfanilamide-treated guinea pig. Lungs were clear in both instances, and the regional lymph nodes were equally involved, enlarged and slightly caseous. The tubercles on the surface of the liver were fewer, and the spleen was smaller, in the treated animal than in the control.

Set 2: The 10 remaining guinea pigs were autopsied 44 days after infection. Macroscopic examination revealed no significant difference between the treated and untreated animals, extent of infection being remarkably similar. Tubercles were found in the lungs in several instances. Livers and spleens were all extensively involved. Regional lymph glands were enlarged and caseating in every case.

Microscopic examination revealed no difference in histopathology between treated and untreated animals.

Summary and Conclusion. (1) Sulfanilamide treatment initiated on the day of infection and maintained throughout the experiment failed to affect the course of experimental tuberculosis in the guinea pig. (2) Guinea pigs treated with sulfanilamide before the infection and treated daily thereafter showed no evidence of being benefited by the drug. (3) It is therefore concluded that, under the conditions of our experiments, sulfanilamide is ineffective as a means of protection against infection with the bovine tubercle bacillus in guinea pigs.

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Hemagglutinins of Pneumococcic Antisera.*

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For a number of years it has been known that some pneumococcic strains possess the Forssman antigen and hence produce in the blood of immunized rabbits hemolysins for sheep blood and agglutinins for blood of Group A. According to a recent report¹ this antigen is present in all strains of pneumococci except those of Types IV, VIB, XI, and XXXI. Prominence was given to these facts only recently on account of the current increased use of therapeutic sera derived from rabbits.

We were led to examine the hemagglutinins and hemolysins of all antipneumococcic therapeutic sera available (rabbit and horse), especially in view of recent reports which indicate that horse sera containing strong-titered agglutinins for human blood were responsible for occasional fatal acute hemolytic reactions.^{2, 3, cf. 4} These accidents were due to injection of horse serum Type XIV.

Our own experiments are based on tests done with therapeutic sera administered to patients at the Harlem Hospital. The sera were tested in dilution of 1:50 for hemolysins of sheep blood and for agglutination of human blood of Groups O, A₂, A₁, and B. Hemolysins for sheep blood were tested by adding 1 drop of 50% suspension of washed sheep-cells to a mixture of 0.5 cc of serum-dilution and 0.5 cc guinea-pig complement 1:10. Tests for agglutination were made by mixing in small tubes 2 drops of a 2% washed blood suspension and 2 or 3 drops of serum dilution; readings were made after the tests stood for one hour at room temperature.

* This study received financial support in part from the Blood Transfusion Betterment Association, the Littauer Pneumonia Research Fund of New York University, College of Medicine, and the Metropolitan Life Insurance Company.

¹ Powell, G. H., and Jamieson, W. A., *Proc. Soc. Exp. Biol. and Med.*, 1938, **39**, 248.

² Bullowa, J. G. M., *The Management of the Pneumonias*, New York, Oxford University Press, 1937, p. 316.

³ Finland, M., and Curnen, E. C., *Science*, 1938, **87**, 417.

⁴ Hoagland, C. L., Beeson, P. B., and Goebel, W. F., *Science*, 1938, **88**, 261.

Our results indicate that antipneumococcic rabbit sera of numerous types contain hemolysins for sheep blood and agglutinins with specific action on human blood of Group A. Of 90 rabbit sera of various types, more than half of them—49—contain distinct agglutinins for blood of Group A. The reactions were distinctly stronger on cells of subgroup A_1 than on A_2 so that in each case a suitable dilution could be found which acted only on A_1 and not on A_2 . The incidence of hemolysins for sheep blood was somewhat slightly higher, 54 out of the 90 sera showing complete hemolysis in 1:50. The maximal hemolytic titer was 1:800; the maximal agglutinin titer for A_1 was 1:1000.

Both agglutinins and hemolysins are specifically absorbable by homologous pneumococci. As was to be expected from our knowledge of the specificity of the Forssman antigen the hemolysins for sheep blood and the agglutinins for human blood of Group A are 2 qualitatively distinct antibodies as could be shown by suitable cross-absorption experiments. In this respect our observation is not in agreement with that of Finland and Curnen.³

None of the antipneumococcic horse sera contains hemolysins for sheep blood, but in a number of them, particularly those of Type XIV, agglutinins for human blood of all groups could be demonstrated. The strongest reactions were found in 3 different specimens of Type XIV antisera, but weaker reactions were found in one specimen each of Type VII antiserum, and a bivalent serum, V and VII. The hemagglutinins in Type XIV antisera were specifically absorbable by large quantities of the homologous organism, a result which is in agreement with that of Finland and Curnen.³

The weaker hemagglutinins in the Type VII antiserum could be shown to be a property of the normal serum, since the reactions on human blood could not be absorbed after contact with the homologous organisms.

The surprising element in the results with the Type XIV antipneumococcic horse sera is the fact that these agglutinins reacted somewhat more intensely on bloods of Groups O and A_2 than on blood of Group A_1 , in sharp contrast to the rabbit sera. This could be confirmed by suitable absorptions which show that such sera, after repeated absorption with blood of Group A_1 , still acted distinctly on bloods of groups O and A_2 (see Table I).

Similar effects, but less differentiating were obtained with each of several different specimens of Type XIV antipneumococcic horse sera absorbed with blood A_1 so that it is safe to state that on the

TABLE I.

Type XIV Antipneumococcic Horse Serum Diluted 1:20 Absorbed 4 Times with One-half Volume of Washed Sediment of Group A₁ Blood and Tested for Action on Bloods of O, A₂, and A₁.

O				A ₂		A ₁			
1	2	3	4	5	6	7	8	9	10
± ±	± ±	++	± ±	+	± ±	±	0	tr	0

whole such horse sera are probably not superior to antibodies from other sources which act on bloods O and A₂.^{5, 6}

Since these therapeutic sera are concentrated and antibodies specific for O and A₂ may be a property of normal animal sera, it was necessary to establish whether or not the specific action on O and A₂ is due to the immunization with pneumococcus XIV. Suitable experiments showed that this organism specifically absorbed the agglutinins prepared by preliminary absorption with blood A₁.

Further evidence as to the specificity of the reaction on bloods of groups O and A₂ is shown in hemolytic tests. Some, but not all, Type XIV antipneumococcic horse sera, when mixed in sufficiently high dilution (1:50 to 1:400) with suitable amounts of guinea-pig complement were found to hemolyze incompletely but distinctly bloods of Group O and to a lesser degree blood A₂ and not bloods A₁ or B. In these instances the hemolytic reactions were accompanied by agglutination. To demonstrate specific hemolysis it was necessary to select guinea pigs whose sera contained no normal hemolysins or agglutinins for bloods of Groups O and A. No hemolysis was observed when fresh human serum was used as a source of complement.

These results recall the observations made on the varying antigenic response of the Forssman antigen of the Shiga bacillus in the rabbit (non-Forssman type) and goat (Forssman type, like the horse). Thus, the Shiga bacillus produces in the rabbit hemolysins for sheep blood (and presumably agglutinins for blood of Group A), but in the goat (Eisler⁷) the same organism induces a response which so far as the hemagglutinins are concerned cannot be differentiated from that observed in Type XIV antipneumococcal horse serum, *i.e.*, agglutinins for human blood of all groups, and in addition another agglutinin specific for O and A₂.⁶ It is also conceivable

⁵ Landsteiner, K., and Levine, P., *J. Immunol.*, 1929, **17**, 1.

⁶ Landsteiner, K., and Levine, P., *PROC. SOC. EXP. BIOL. AND MED.*, 1930, **28**, 309.

⁷ Eisler, M., *Z. f. Immunitätsf.*, 1930, **67**, 38.

that an explanation for the strikingly varying behavior of antisera derived from the horse and goat in contrast to those from the rabbit may be found in a difference in their essential lipoids (Horsfall and Goodner).⁸

In view of the observations recorded, it is pertinent to inquire about the blood group of the individuals who died from the administration of the therapeutic horse sera. Unfortunately, these data are not available.

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Pneumonia in White Mice Produced by a Pleuro-Pneumonia-Like Micro-Organism.*

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During the past year, while working with tissues and exudates from patients with rheumatic fever or rheumatoid arthritis, a pleuro-pneumonia-like micro-organism has been encountered in our laboratory mice.

In each instance, normal young white mice of the same breed were inoculated intranasally under ether anesthesia with 0.05 cc of 10-20% tyrode or saline suspensions of human pathologic tissues. However, exudates were introduced without dilution. Serial mouse-passage was carried on at intervals of 4-6 days, using 10-20% lung-suspensions. Blind passages were done in a parallel fashion. Usually by the fourth passage, purple areas of pneumonic consolidation were clearly visible in one or more lobes. In one instance, the pneumonia appeared as early as the second passage. Further passage slowly increased the virulence, morbidity and mortality. Even after months of passage, however, the mortality never increased beyond 20-30%, with death usually occurring on the fourth or fifth day.

Culture of the ground lungs uniformly grew innumerable colonies

⁸ Horsfall, F. L., and Goodner, K., *J. Immunol.*, 1936, **31**, 135.

* The expenses of this investigation were defrayed in part by the Commonwealth Fund.

This is publication Number 31 of the Robert W. Lovett Memorial for the study of crippling disease, Harvard Medical School.

of a pleuro-pneumonia-like micro-organism. The morphology and nature of this microbe has been described in a previous note.¹ Other bacteria were rarely encountered, and these usually disappeared with the next passage. However, in 2 instances, the mortality was highly increased by the association of a gram negative bacillus.

The infective agent was readily preserved by freezing at -80°C . It grew nicely on boiled-blood-ascitic agar plates or broth, in as wide a pH range as 7.0 to 7.8. Multiplication occurred in chick-embryo-tyrode tissue-culture medium,² or on embryo-tyrode-agar.³ We were totally unsuccessful in attaining growth upon the chorio-allantoic membranes of chick embryos in the standard fashion. However, if the inoculated embryo was chilled to death at 4°C , followed by incubation, growth and serial passage were readily accomplished. Organisms grown in this fashion, however, appeared to lose their virulence. Dr. Sabin informs us that two of our strains examined by him are serologically identical with his strain A.^{4, 5}

When a large number of mice were simultaneously inoculated with a suspension of the same infective material, it was observed that during the first day there was little change in their clinical appearance except for slight ruffling of the fur. However, even by the end of the first 24 hours, there were small 1-2 mm purple consolidated areas close to the hilar great vessels. Sickness progressed rapidly, with failure to eat or drink, and loss of weight. Some became markedly dyspnoeic. These began to die about the third day, and exhibited almost total pulmonary consolidation. Microscopically, the pneumonia was largely interstitial, with mononuclear phagocytes the dominant cell. Some of these were in mitosis, others actively phagocytic, and a few by fusion formed large giant cells. The consolidated areas were congested. Polymorphonuclear leukocytes were present, especially numerous within the small bronchi. There were a few areas of compensatory alveolar emphysema.

By the seventh day, one of the mice showed a pleuritis. Following this, the survivors became less sick, and began to gain in weight. Periodic autopsy, however, disclosed that some showed small pneumonic areas as late as the twentieth day. On the twenty-sixth day, the entire left lung of one mouse had become pearly gray and cystic in appearance, and the cut surface exuded sticky mucoid material.

1 Dienes, L., and Sullivan, E. R., *Proc. Soc. Exp. Biol. and Med.*, in press.

2 Li, C. P., and Rivers, T. M., *J. Exp. Med.*, 1930, **52**, 465.

3 Zinsser, H., Fitzpatrick, F., and Wei, H., *J. Exp. Med.*, 1939, **69**, 179.

4 Sabin, A. B., personal communication.

5 Sabin, A. B., *Science*, 1938, **88**, 189, 575; 1939, **89**, 228.

Similar lesions occur in rats naturally infected with pleuro-pneumonia-like organisms.

When injected intravenously, intraperitoneally or subcutaneously in the two strains of mice at our disposal, our cultures failed to produce any clinical-pathological phenomena. As has been previously noted,⁶ however, the susceptibility of different strains of mice is very variable.

Although we encountered this disease and this microbe while working with human rheumatic material, we do not feel justified in concluding that our agent came from aught except mice. Our reasons are these. A similar strain was secured by blind passage of mouse lungs. Using appropriate media, we have been constantly unable to grow it directly from human pathological material, even from uncomplicated fatal active rheumatic autopsy material. Yet such material, when passed through mice, readily yielded the organism.

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Wheat Germ Oil and Tumor Formation.

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Rowntree¹ reported the occurrence of sarcoma in rats fed on a diet containing wheat germ oil, all his animals developing tumors after treatment for 15-111 days (54 days on the average) with a daily dose of 4 cc of the oil mixed with their normal food. The ability of the oil to produce tumors depended on the method of extraction, the extract with ether having cancer-producing properties, while that obtained with petroleum ether appeared to be inactive. A repetition of these experiments of Rowntree seems extremely important, because if they could be confirmed it should be possible by slightly modifying the method of extraction to obtain very highly purified preparations. Assuming that the active substance is soluble in ether but not in petroleum ether, extraction of wheat germs with the latter solvent should remove all the fats and

⁶ Dienes, L., and Edsall, G., *PROC. SOC. EXP. BIOL. AND MED.*, 1937, **36**, 740.

¹ Rowntree, L. G., Steinberg, A., Dorrance, G. M., and Ciccone, E. F., *Am. J. Cancer*, 1937, **31**, 359.

TABLE I.

Preparation	Quantity per rat per day in cc	No. of days	Total quantity per rat in cc
I. Ether extract from wheat germs, according to Rowntree	3.4	267	817
II. Petroleum ether extract	4.5	73	297
III. Ether extract of the portion in- soluble in petroleum ether	4.5	89	361
IV. Wheat germ oil from pressed wheat germs	3.4	291	889
V. Crude ether extract not freed from fatty acids, etc.	4.5	89	361
VI. Olive oil	4.5	73	297

phosphatides, *i. e.* more than 95% of the lipid fraction, and subsequent treatment with ether should give a solution of the active substance in a highly purified state.

Six groups of 10 rats resulting from a cross-breeding between Piebald and Wistar strains were used. Each group consisted of 7 males and 3 females accommodated in separate iron cages. The initial weights lay between 130 and 342 g. The room temperature was fairly constant between 20-22°C. The ground food, which was an accurate copy of that described by Rowntree, was mixed with the various extracts and the quantity was so chosen that all food was completely consumed.

Practically all the rats remained in excellent condition and increased regularly in weight. Dissection took place 73 to 291 days after the beginning of the test. The extracts used, the time during which they were applied and the total amounts given, are summarized in Table I.

Large tumors like those described by Rowntree were never observed in a single case. All the tissues in the abdomen which showed an abnormal appearance were examined histologically, but no tumor, neither sarcoma nor carcinoma, could be established. The histological examination was checked by the carcinologist, Dr. Korteweg, and found to be correct.

According to recent communications, Day and his coworkers,² Carruthers³ and Halter⁴ have also been unable to induce sarcoma in rats with ethereal extracts of wheat germs.

Summary. The experiments of Rowntree and associates have been repeated as accurately as possible but their results could not

² Day, H. G., Becker, J. E., and McCollum, E. V., *PROC. SOC. EXP. BIOL. AND MED.*, 1939, **40**, 21.

³ Carruthers, C., *Ibid.*, 1939, **40**, 107.

⁴ Halter, C. R., *Ibid.*, 1939, **40**, 257.

be confirmed. Not a single case of sarcoma was observed in 2 groups of 10 rats after administration of ether extracts of wheat germs in large quantities over a long period (5 times as long as is necessary according to Rowntree). Two groups of 10 rats treated with wheat germ extracts made in other ways and one group of 10 rats treated with crude press oil from wheat germs also failed to develop sarcoma.

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Correlation Between Secretion of Dyestuffs by the Kidney and Molecular Structure of These Dyes.*

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Very little is known about the mechanism of the active secretory transport of the kidney. We have tried to attack this problem by investigating the secretory activity of the tubules of the frog kidney as regards dyestuffs, which more or less resemble one another by their molecular configuration. The isolated kidney was perfused with Ringer solution through the aorta under a pressure of about 24 cm of water and with Ringer solution containing 0.0005% of dyestuff through the renal portal vein under a pressure of about 12 cm. About 30 dyestuffs have been tested, all of them being mono-azo-sulfonic acid dyes and all of them being diffusible.

One series of experiments was concerned with 8 naphthalene-azo-naphthalene-disulfonates. The result obtained showed an obvious connection between the structure of the dye and its aptitude for secretory concentration. The main decisive feature is the location of the sulfonate groups in the molecule. If both sulfonates are on the same half of the molecule, as with Fast Violet R, Echtrot B, Acid Violet 6R and Palatine Red A, the injected dye reappears in the secretion at a higher concentration. If one sulfonate is attached to one naphthalene nucleus, the other sulfonate to the other, as

* Supported by a grant from the Penrose Fund of the American Philosophical Society and by a grant from the Ella Sachs Plotz Foundation. Dyestuffs were supplied by E. I. du Pont de Nemours & Company, by I. G. Farben Aktiengesellschaft, and by National Aniline and Chemical Company.

in Fast Red C, Fast Red E, Serichrome Blue R and Brilliant Ponceau 4R, little or no secretion occurs. As yet, only one exception has been met, Crocein Scarlet 3BX. This dye is secreted although the 2 sulfonate groups are in opposite positions. This exceptional behavior could possibly arise from the particular location of one sulfonate with respect to the azo group. We shall come back to this point later.

In another series of experiments, 13 benzene-azo-naphthalene-sulfonates were used. Eight of them were mono-sulfonates, 5 of them di-sulfonates. In 3 of the mono-sulfonates, Azofuchsin B, Orange GT and Brilliant Orange R, the sulfonate group is attached to the naphthalene ring system, while in 5 of them, Tropaeolin 000/2, Tropaeolin 000/1, Orange R, Superchrome Violet B and Lithosol Rubine B, it is attached to the benzene ring. All these benzene-azo-naphthalene-mono-sulfonates undergo secretory transport.

Of 5 disulfonates used, 4 were secreted, one was not. These 4 are Ponceau R, Palatine Scarlet A, Azofuchsin I and Azofuchsin II. Here, both sulfonates are located on the naphthalene nucleus. The one non-secreted dye, Azofuchsin G, provided the chance of a crucial experiment in this series of dyestuffs. Since each sulfonate is attached to one half of the molecule, there is no active transport.

So far, we come to the conclusion that, with 20 dyestuffs from a collection of 21 of them, the location of the sulfonate group in the molecule is the controlling factor for physiological behavior. The following explanation could be proposed: the common feature in the configuration of our dyestuffs is that their basal structure is composed of two halves. If sulfonate groups are attached only to one half, the result is a polar-nonpolar configuration, the sulfonated half being hydrophilic, the other half hydrophobic and organophilic. This configuration would enable the molecule to anchor at the interface between cell and surroundings as a first step of penetration. Sulfonate groups, however, fixed on both halves, would prevent the molecule from being attached therein.

Greater difficulties have been encountered in studying the behavior of mono-azo-dyestuffs with 3, 4 or 5 sulfonate groups. We have learned something about some of them, where the sulfonate groups are irregularly disposed around the molecule (Ponceau 6R, I.G.Nr.XIV and Azofuchsin V). The kidney fails to pick them up, presumably because their hydro-affinity is overwhelming. A series of 5 isomers of the naphthalene-azo-naphthalene tri-sulfonates

is particularly interesting. Three of them, Fast Wool Blue R, Fast Wool Blue B and Amaranth were not secreted, while 2 of them, Scarlet RR and Chromotop 8B were. These 2 are comparable to the disulfonate Crocein Scarlet 3BX, which was mentioned before as outstanding by a special disposition of one sulfonate group, which might be supposed to change the intramolecular forces concerned.

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Effects of Anterior Pituitary and Adrenal Cortical Extracts on Metabolism of Adrenalectomized Rats Fed Glucose.

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Whole anterior pituitary extracts given to normal rats fed glucose prevent the usual elevation of R.Q. and increase deposition of muscle glycogen.^{1, 2}

Adrenal cortical extracts,³ corticosterone, and certain adrenotropic anterior pituitary extracts have now also been shown to diminish the rise in R.Q. and to promote glycogen deposition. These findings suggested that part or all of the action of the anterior pituitary extract in fed animals might be mediated through the adrenal cortex. A study has therefore been made of the relative effects of anterior pituitary extract and of adrenal cortical extract (CE) on the disposition of fed glucose in the absence of the adrenal glands.

The experiments were carried out as described previously:² young male rats were fasted 18 hours, then fed known amounts of glucose, the respiratory data was obtained during the 4-hour period after feeding, and terminal analyses were made of liver and muscle glycogen, blood glucose and glucose remaining in the gastro-intestinal tracts. Recovery and oxidation of the fed glucose are presented here, calculated as percent of the absorbed amounts. The figures are averages of 9 or 10 experiments in each group.

* National Research Council Fellow in Medicine, 1938-39.

¹ Meyer, H. S., Wode, L. J., and Cori, C. F., *PROC. SOC. EXP. BIOL. AND MED.*, 1937, **36**, 346.

² Russell, J. A., *Am. J. Physiol.*, 1938, **121**, 755.

³ Katzin, B., and Long, C. N. H., *Proc. Am. Physiol. Soc.*, 1938.

The rats were adrenalectomized from 3 to 20 days before the experiments and were maintained in good condition in the interim by the administration of saline drinking water. All carbohydrate levels were low in the fasted adrenalectomized rats; the muscle glycogen values, however, were still much above those of fasted hypophysectomized rats. The glucose absorption rates under these conditions were also reduced moderately, but again not to the same extent as in hypophysectomized rats.

The extracts used in these experiments were a 2% saline extract of beef anterior lobes clarified by Sharples centrifugation, and Upjohn's cortical extract from which the alcohol had been removed by vacuum distillation. One ml of the anterior pituitary extract, containing about 10 mg organic solids, was given intraperitoneally 1 to 1½ hours before the glucose was fed. The cortical extract was given in total dosages of 3 to 5 ml, in part injected intraperitoneally ½ to 1 hour before the glucose feeding, and in part injected or fed with the glucose.

Five series of experiments are summarized in the accompanying table: those carried out on untreated adrenalectomized rats, on adrenalectomized animals treated with anterior pituitary extract alone, with cortical extract alone, and with a combination of the 2 extracts, and on normal controls. In the untreated adrenalectomized rats the significant changes in the disposition of fed carbohydrate were: greater increases in the glucose found in body fluids, probably in part because of the low initial levels, and moderate reduction in the storage of liver glycogen (an average value of 1.5% liver glycogen was obtained). The results are in contrast to those observed in hypophysectomized rats where very much more carbohydrate was oxidized.²

TABLE I.
Disposition of Fed Carbohydrate in Adrenalectomized Rats.
% of glucose absorbed in 4 hours.

	Glucose recovered after 4 hr			Glucose Oxidized	Total Accounted for
	In body fluids	As muscle glycogen	As liver glycogen		
A. Adrenalectomized rats					
1. Untreated	6±0.5*	10±3	9±1	55±5	80
2. A.P.E.†	9±1.3	14±2	4±1	56±3	83
3. C.E.‡	9±0.5	13±2	16±2	41±3	79
4. A.P.E. and C.E.	6±0.5	35±2	13±1	29±3	83
B. Normal rats	3±0.2	14±1	17±1	49±2	83

* Standard error.

† A.P.E.: 1 ml saline extract 1 to 1½ hours before glucose feeding.

‡ C.E.: 3-5 cc Upjohn's cortical extract 0 to 1 hour before glucose feeding.

The anterior pituitary extract was without any effect on the apparent rate of oxidation of fed carbohydrate in the adrenalectomized rats. Its only action was to decrease liver glycogen deposition and to increase somewhat the body fluid glucose and perhaps muscle glycogen. The cortical extract, on the other hand, in the doses given brought about moderate reduction in the apparent rate of oxidation of the fed carbohydrate, and increased markedly the liver glycogen deposition. But the depressing effect of anterior pituitary extract on carbohydrate oxidation was not strictly an adrenotropic effect as shown by the results of series 4. In these experiments, when anterior pituitary extract and cortical extract were given at the same time to adrenalectomized rats, the anterior pituitary extract was able to exert its usual full effect: there was a marked reduction of the oxidation rate beyond that produced by cortical extract alone, and the excess carbohydrate was mainly deposited as muscle glycogen.

The effects of these hormones on the disposition of fed carbohydrate, while superficially similar, are therefore to be distinguished. First, in adrenalectomized as in normal rats, when cortical extract is diminishing net carbohydrate utilization it increases liver glycogen but not peripheral tissue glycogen levels. Anterior pituitary extract, on the other hand, increases muscle glycogen stores but not those of the liver in these animals. Further, anterior pituitary extract, which produces a more intense depression of carbohydrate oxidations in normal rats, requires for this action the presence of some cortical hormone, but not the active mediation of the adrenal cortex itself. That is, there appear to be in the circumstances of these experiments both complementary and synergistic relationships between the metabolic activities of the cortical and anterior pituitary hormones.

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Action of Gonadotropic Hormones in Amenorrhea as Evaluated by Vaginal Smears.*†

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We reported that the vaginal smear could be used in women to evaluate the action of estrogens, adequate amounts of which transformed the menopausal smear to the follicular type seen during the normal menstrual cycle at the height of follicular activity.¹ Full replacement therapy could, by its use, be insured not only in the menopause but in amenorrhea as well. Its value for the biological assay of estrogens in the human was pointed out. Subsequent studies from this laboratory demonstrated the applicability of the smear to the analysis of the action of androgens in women.² They were shown to be capable of suppressing menstruation, transforming the normal smear to an atrophic type seen in the menopause and primary amenorrhea. It was also found that androgens, given to menopausal women receiving full replacement therapy with estrogens, caused a regression of the induced follicular smear to the original menopausal level.³ The smear could thus be used to control the therapeutic use of androgens in women wherever indicated.

The present study deals with the application of the smear method to a functional classification of amenorrhea and to the evaluation of another group of hormones, the gonadotropic principles, which are being employed in this condition in an effort to induce ovarian activity.

The status of our present knowledge of the amenorrheas and their therapy is quite unsatisfactory. This is due in part to the lag in chemical progress in the field of the gonadotropic principles, as compared with the estrogens, as well as to our ignorance of the

* This study was aided by a grant from the Josiah Macy, Jr., Foundation and the Committee for Research on Sex Problems of the National Research Council.

† We are indebted to E. R. Squibb and Sons and to the Upjohn Company for their generous supply of Follutein and Gonadogen, respectively.

1 Papanicolaou, G. N., and Shorr, E., *Am. J. Obst. and Gyn.*, 1936, **31**, 806.

2 Papanicolaou, G. N., Ripley, H., and Shorr, E., *PROC. SOC. EXP. BIOL. AND MED.*, 1938, **37**, 689; *Endocrinology*, 1939, **24**, 339.

3 Shorr, E., Papanicolaou, G. N., and Stimmel, B., *PROC. SOC. EXP. BIOL. AND MED.*, 1938, **38**, 759.

exact mechanisms underlying the production of the amenorrheas.

A more satisfactory classification of this condition than now exists should help clarify this problem. It is customary to divide the amenorrheas into two groups. If menstruation has never occurred, they are classified as "primary." If amenorrhea has interrupted a period of cyclic menstrual activity, it is considered as the "secondary" type. Examination of the vaginal smears of approximately 40 such patients makes it clear that such a classification is too static, and might well be supplemented by a functional one, based on the degree of associated ovarian under-function as well as the presence or absence of cyclic ovarian activity. On the basis of the smear picture, the amenorrheas can be subdivided into 3 groups. The first is characterized by the constant presence of the atrophic smear which we have interpreted as indicative of virtual absence of ovarian activity.¹ This group includes most "primary" and many "secondary" amenorrheas. The second group exhibits smears which are quite constant for each case and are indicative of some degree of subnormal and uniform ovarian activity. Into this group fall most of the "secondary" amenorrheas. The third and smallest group shows irregular cyclic smear changes, imitating those seen during the normal menstrual cycle, and reflecting periodic ovarian activity, which is however insufficient to produce overt menstruation. This picture is seen chiefly in "secondary" amenorrheas and in adolescents with delayed puberty.

With such a variety of types, the present confusion as to the effectiveness of available gonadotropic agents might be anticipated. Our own experience as to the unpredictability of the response to this group of hormones agrees with that of other workers. However, sufficient positive results have been obtained to indicate the potential value of the gonadotropic hormones and the usefulness of the smear method in their evaluation.

The changes induced in the smears in the amenorrheas, by these hormones, range from a brief congestive response, marked chiefly by an increased secretion of mucus, with little or no change in the cellular picture, through intermediate stages to the development of a full set of changes comparable to those seen in the course of a normal menstrual cycle. For the sake of brevity, the smear changes induced in one such case by a gonadotropic extract will be described in detail as exemplifying a fairly complete type of ovarian response to these hormones.

Case History: Age 19, single, in good health. Catamenia at 14. For first 6 months irregular, at 2-5 week intervals, then regular,

at 4 week intervals, lasting 2-4 days, and somewhat scant until 18 years when they ceased. Duration of amenorrhea was 15 months, except for 4 months during which mixed hormonal therapy including thyroid, estrone, and pregnancy urine extract resulted in three bleedings, the last 4 months prior to admission to Endocrine Clinic.

Control Vaginal Smears: Smears taken for a month prior to treatment were of the atrophic type (Figure 1) we have described as occurring in most "primary" amenorrheas and many castrates.¹ We interpret this smear as indicating profound depression of ovarian activity.

Treatment: Pregnancy urine extract (Follutein) was given as follows: beginning 12/6/38 with 20 R.U. subcutaneously, the extract was given daily for 9 days in increasing doses with a final dose of 1,000 R.U. on 12/15/38 (Total = 4,470 R.U.)

Vaginal Smears: On 12/12-13/38 there was a strong mucous reaction, the cellular picture remaining unchanged. On 12/18-19/38 mucus was again seen together with some fibrination and a few erythrocytes. From 12/28 on there occurred a gradual change in the smear picture with the development of a follicular type which persisted from 1/7-13/39 (Figure 2). Slight microscopic bleeding was seen from 1/7-11/39. Early regression was apparent on 1/15/39 and more advanced by 1/17/39 (Figure 3). There was no distinct premenstrual phase. On 1/18-20/39 moderate bleeding appeared (Figure 4). The early appearance of the bleeding and the lack of a typical premenstrual phase were interpreted as indicating the absence of ovulation. During the bleeding phase (1/18-20/39) deep cells reappeared, increased in number, and the smears changed to their original atrophic character. The patient reported a faint show on 1/8-10/39 and on 1/18-20/39 what she considered an entirely normal menstrual flow.

Second Course of Treatment: Ten injections of Follutein in increasing amounts (50-1,000 R.U. totalling 3,760 R.U.) were given from 2/1-14/39.

Vaginal Smears: The initial level was typically atrophic. After an initial mucous reaction the cell type changed gradually to reach a follicular phase on 2/10/39. Regression began 2/14/39 and was slower than before. Bleeding was seen in the smears of 2/22-24/39 which were a little more typical of the normal menstrual smear. Deep cells reappeared in large numbers on 2/25/39 and persisted. The patient reported what she considered a normal menstrual flow on 2/21-24/39.

Third Course of Treatment: This time a gonadotropic extract

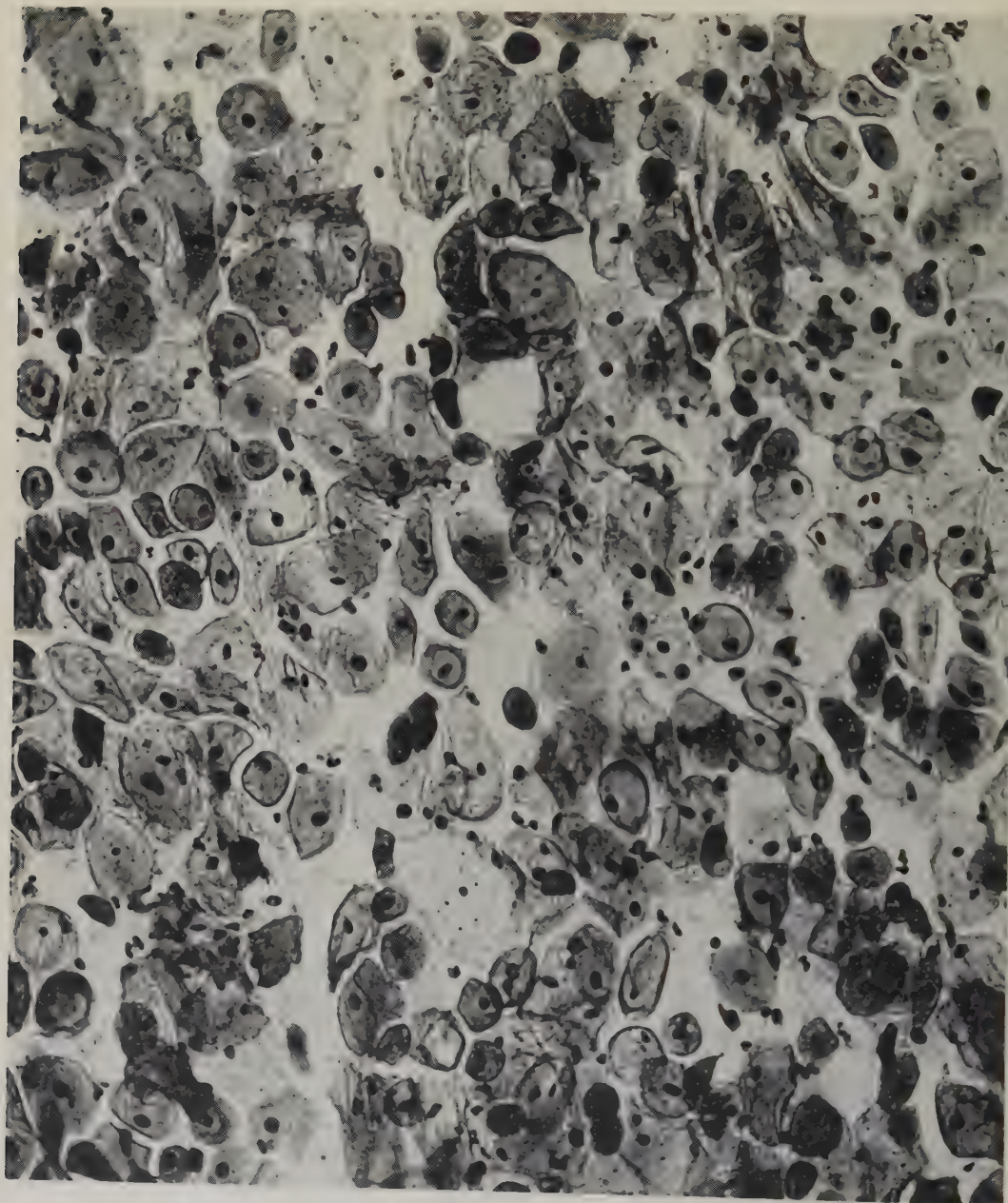


FIG. 1.
Original type of atrophic vaginal smear, showing many deep cells. $\times 250$.
from pregnant mare serum (Gonadogen) was used, 150 units being
given intravenously on 3/11/39.

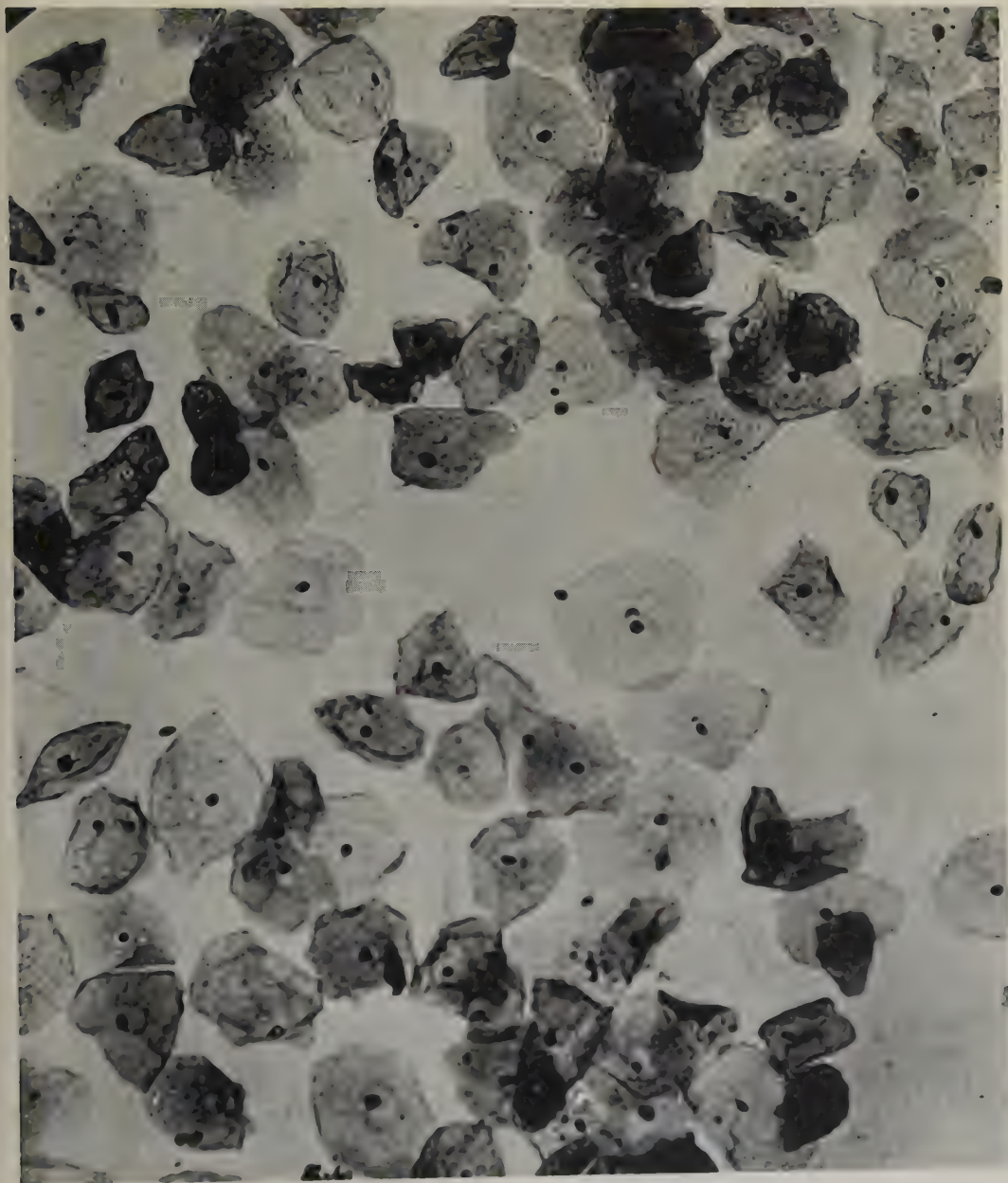


FIG. 2.
Follicular type of vaginal smear, induced by treatment. $\times 250$.

Vaginal Smears: An early or pre-follicular phase was reached on 3/18-19/39, followed by a rapid regression with reappearance

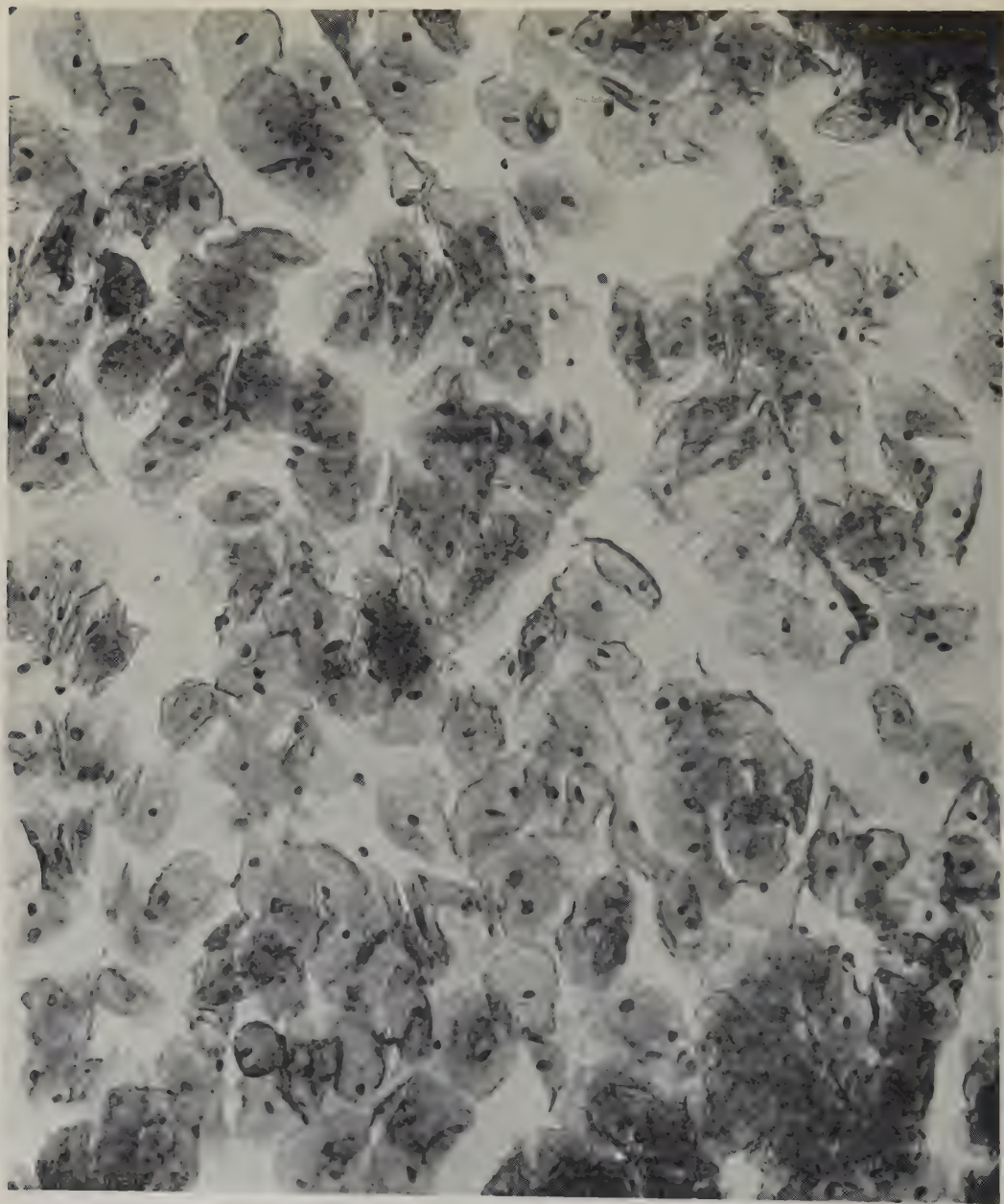


FIG. 3.
Vaginal smear showing regression. $\times 250$.

of deep cells and microscopic bleeding 3/21-22/39. There was no overt bleeding.



FIG. 4.
Vaginal smear showing bleeding. $\times 250$.

Fourth Course of Treatment: Another course of Follutein was given 4/11-24/39. Initial dose 50 R.U., final dose 1,000 R.U. (Total 4,950 R.U.)

Vaginal Smears: Control smears were less atrophic than the original ones. Starting 4/12/39 a gradual change to an early follicular smear occurred which was maximal 4/22/39. The regression proceeded at a slower rate with very few deep cells, and the smears were nearer the normal premenstrual type. Bleeding was seen on 5/3-6/39 with a few deep cells reappearing towards the end of the flow. The patient reported a normal menstrual flow on 5/3-6/39.

Comment: This case we would now classify as a secondary amenorrhea of the stationary or "fixed" atrophic type. This type, in our experience, rarely resumes ovarian activity spontaneously. For this reason we feel that the changes in ovarian activity as reflected in the vaginal smears were actually due to the treatment given. Another reason for this belief is that following each course of treatment the smears reverted to the original atrophic type.

The degree of ovarian stimulation would appear to have been considerable since smear changes of a follicular type were induced, quite comparable to those seen during the normal menstrual cycle. Furthermore, the estrogen elaborated was sufficient to build up an endometrium from which there was a flow equal in amount and duration to the patient's normal flow. Whether true ovulation occurred is questionable and it is quite likely that the first 3 cycles, at least, were anovulatory. The post-follicular smears in these cycles were not of the usual premenstrual character but rather like those seen on estrin withdrawal in amenorrhea or the menopause, where the corpus luteum hormone does not influence the smears and bleeding occurs in 5-6 days after treatment stops. The post-follicular smears of the 4th cycle were more premenstrual in character, the bleeding occurred 12 days after the follicular stage, and the menstrual smears were more normal. This cycle may have been accompanied by ovulation.

Summary: The desirability of a reclassification of the amenorrheas based, in part, on the vaginal smear picture has been suggested. Definite smear changes indicative of ovarian stimulation and resembling the normal cycle have been induced in amenorrhea by means of gonadotropic hormones. The vaginal smear should afford a simple and sensitive method for evaluating the effects of such hormones in the human subject.

Acetone Fractionation of Blood and Urinary Iodine.

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Correlation of the forms in which iodine exists in the blood with those found in the urine is of significance in determining the fate of thyroxine in the human body. Barkan¹ separated the "organic" iodine of the blood and urine from the "inorganic" by precipitation with silver nitrate. He reports that from 20 to 70% of the total iodine in normal urine is organically bound. Foster and Gutman² fed diiodotyrosine to rabbits and found 10% of the urinary iodine as inorganic iodide, 60% as unchanged diiodotyrosine, 18% as 3,5-diiodo-4-hydroxyphenyllactic acid, and 12% as iodine lost in the separation. Eufinger and Schulte³ employed an 80% acetone solution for the separation of "organic" and "inorganic" iodine in the blood.

We have employed a 75% acetone solution in fractionating both the blood and urinary iodine. The urine specimens were collected in 3-day (72-hour) periods. The blood was drawn and the B.M.R. determined on the second day of each period. The periods studied were: I. Normal, nonconstant regimen with no iodine medication; II. Normal, nonconstant regimen with 10 mg of iodine as KI given daily for 7 days—the last 3 days being the period of study; III. Normal, nonconstant regimen with 3 grains of desiccated thyroid given daily for 18 days—period of study covering the 18th, 19th and 20th days after starting the thyroid medication. All medication was given orally. The subject was a normal male.

One hundred ml of urine is treated with 300 ml of 99.5% iodine-free acetone. The mixture is shaken well and allowed to stand over night. The precipitate is then filtered off and washed well with acetone. The filtrate and washings are analyzed together for iodine and termed the acetone-soluble fraction. The precipitate is then washed with 500 ml of double-distilled water, which is analyzed for iodine and termed the acetone-insoluble but water-soluble fraction. The remaining precipitate is then analyzed for iodine and termed the acetone and water-insoluble fraction.

* William Wallace Kincaid Fellow in Research Surgery.

¹ Barkan, G., *Arch. f. Exp. Path. und Pharmacol.*, 1928, **138**, 160.

² Foster, G. L., and Gutman, A. B., *J. Biol. Chem.*, 1930, **87**, 289.

³ Eufinger, H., and Schulte, W., *Arch. f. Gynak.*, 1933, **152**, 479.

TABLE I.
Urinary Data for the Three Periods of Study (3 days per period).

Day	Period I			Period II			Period III		
	No Medication			10 mg Iodine as KI			3 grs Desiccated Thyroid		
	B.M.R. % —8			B.M.R. % —8			B.M.R. % +5		
	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd
Total urinary volume (ml)	950.0	690.0	860.0	750.0	800.0	675.0	1640.0	1345.0	1340.0
Total iodine output (gamma*)	231.0	97.3	131.0	730.0	542.0	682.0	445.0	291.0	192.0
" (gamma %)	24.3	14.1	15.2	97.3	67.7	101.0	27.1	21.6	14.3
Acetone-soluble iodine (gamma %)	24.1	13.5	15.6	96.5	66.9	100.2	26.3	20.5	13.1
Acetone-insoluble-water-soluble iodine (gamma %)	0.20	0.42	0.10	0.67	0.61	0.58	0.31	0.21	0.35
Acetone- and water-insoluble iodine (gamma %)	0.0	0.0	0.0	0.15	0.11	0.16	0.0	0.0	0.0

* 1 gamma = 1 microgram = .001 mg.

TABLE II.
Blood Iodine Data for the Three Periods of Study.
(One blood analysis per period—blood drawn on 2nd day of period.)

	Period I		Period II		Period III	
	No Medication		10 mg Iodine as KI		3 grs Desiccated Thyroid	
	B.M.R. % —8		B.M.R. % —8		B.M.R. % +5	
Acetone-soluble iodine (gamma %)	1.50		5.33		1.94	
Acetone-insoluble-water-soluble iodine (gamma %)	0.93		1.10		2.60	
Acetone- and water-insoluble iodine (gamma %)	1.20		1.10		1.36	
Total iodine (gamma %—calculated from fractions)	3.63		7.53		5.90	

The above procedure was used in the blood fractionation except that 50 ml of fresh, untreated blood and 150 ml of acetone were employed.

The Matthews, Curtis and Brode⁴ modification of the Leipert procedure was employed in determining the iodine content of the 3 fractions.

The results of the urine analyses for the 3 periods studied are given in Table I. It will be seen from this table that all of the urinary iodine is acetone-soluble regardless of the form of iodine ingested. The results of the blood analyses for the 3 periods of study are given in Table II. From the data presented in this table a definite variation in the iodine content of the fractions will be noted. This appears to be due to the form of iodine ingested. From these data it would appear that the acetone-soluble fraction contains that portion of the blood iodine eventually excreted in the urine and that the other 2 blood fractions contain the iodine that is not excreted in that form by the kidneys. These two fractions apparently contain iodine in a different form than the urinary iodine. It is not to be assumed, however, that all of the iodine excreted in the urine or found in the acetone-soluble fraction of the blood exists in a form that cannot be utilized by the body.

It is of interest to note the dominance of the acetone-soluble fraction of iodine in the normal blood as well as after potassium iodide medication, whereas after thyroid medication the acetone-soluble fraction falls, while the acetone-insoluble but water-soluble fraction appears to increase. The significance of the variation of these blood iodine fractions is as yet unknown.

Summary. 1. All of the urinary iodine appears to be acetone-soluble. 2. There is an acetone-soluble; and acetone-insoluble but water-soluble; and an acetone- and water-insoluble form or forms of iodine in the blood. 3. The quantitative relationship between the iodine fractions of the blood varies with the form of iodine ingested. 4. The acetone-soluble fraction of the blood iodine may possibly contain the iodine compounds which will later be excreted in the urine. 5. The true significance of these fractions is as yet unknown.

⁴ Matthews, Norman L., Curtis, George M., and Brode, Wallace R., *Ind. and Eng. Chem., Anal. Ed.*, 1938, **10**, 612.

Effect of Adrenal Insufficiency on Distribution of Chlorides Between Plasma and Erythrocytes.

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Lucas¹ was probably the first to observe a decrease of the plasma chloride concentration in the suprarenalectomized dog. Since then, numerous other authors confirmed his findings in various species of experimental animals.²⁻¹² Although several investigators obtained negative or contradictory results,¹³⁻¹⁶ it is now a generally accepted fact that adrenal insufficiency results in a decrease in blood chlorides.

In the course of our previous studies on the effect of various stimuli on blood chlorides,¹⁷ we found that the red cell chloride concentration is usually a more sensitive test of beginning hypochloremia than the whole blood or plasma chloride concentration. Since most of the above mentioned investigators used whole blood, plasma or serum for their determinations, it appeared of interest, therefore, to establish whether this would also be true in the case of hypochloremia

¹ Lucas, G. H. W., *Am. J. Physiol.*, 1926, **77**, 114.

² Rogoff, J. M., and Stewart, G. N., *Am. J. Physiol.*, 1926, **78**, 711.

³ Baumann, Emil J., and Kurland, Sarah, *J. Biol. Chem.*, 1927, **71**, 281.

⁴ Swingle, W. W., *Am. J. Physiol.*, 1928, **86**, 450.

⁵ Zwemer, R. L., and Sullivan, R. C., *PROC. SOC. EXP. BIOL. AND MED.*, 1931, **28**, 723.

⁶ Ohguri, Minoru, *Tohoku J. Exp. Med.*, 1931, **17**, 390.

⁷ Loeb, Robert F., Atchley, Dana W., Benedict, Ethel M., and Leland, Jessica, *J. Exp. Med.*, 1933, **57**, 775.

⁸ Harrop, George A., Weinstein, Albert, Soffer, Louis J., and Trescher, John H., *J. Exp. Med.*, 1933, **58**, 1.

⁹ Harrop, George A., Soffer, Louis J., Ellsworth, Reed, and Trescher, John H., *J. Exp. Med.*, 1933, **58**, 17.

¹⁰ Harrop, George A., Nicholson, W. M., and Strauss, M., *J. Exp. Med.*, 1936, **64**, 233.

¹¹ Britton, S. W., and Silvette, H., *Am. J. Physiol.*, 1937, **118**, 594.

¹² Harrison, Harold E., and Darrow, Daniel C., *J. Clin. Invest.*, 1938, **17**, 77.

¹³ Pannella, Pasquale, *Riv. Pat. sper.*, 1930, **6**, 132.

¹⁴ Urechia, C. L., *Gr. Benetato et Retezeanu: Bull. Acad. Méd. Roum.*, 1936, **1**, 141.

¹⁵ Malaguzzi, Valeri C., *Arch. di Sci. biol.*, 1935, **21**, 79.

¹⁶ Simpson, S. Levy, Dennison, M., and Korenchevsky, V., *J. Path.*, 1934, **39**, 569.

¹⁷ Karady, S., Selye, H., and Browne, J. S. L., *Proc. Am. Physiol. Soc.*, Toronto, April, 1939.

induced by adrenalectomy. For this purpose, we removed the adrenals from 10 male "hooded" rats weighing 160-190 g. They received food and water *ad libitum* during the first 24 hours after the operation. Then food was withdrawn for 24 hours so as to eliminate the possible effect of varying food intake. At the end of this fasting period, these and 24 control animals of the same strain, weight and sex and fasted for the same length of time were killed. Their blood was collected, clotting being prevented by sodium oxalate. The chlorides were then directly determined in the plasma and the whole blood while red cell chlorides were calculated from these

TABLE I.
Chloride Concentrations Are Expressed in mg of Chloride per 100 cc of Material.

No.	Hematocrit	Whole Blood Chlorides	Plasma Chlorides	Red Cell Chlorides	Cl _c :Cl _{pl}
Normal Animals.					
1	40	298	369	189	.51
2	41	291	362	188	.52
3	43	277	355	175	.50
4	40.5	284	355	180	.50
5	42	284	362	174	.50
6	42	291	362	184	.50
7	41.5	284	362	174	.49
8	42.5	277	355	172	.49
9	42	284	362	174	.50
10	41	291	369	192	.52
11	42.5	277	348	181	.52
12	42	291	376	183	.49
13	42	284	369	172	.48
14	41.5	291	362	190	.52
15	42	298	376	190	.50
16	43	284	362	182	.50
17	42	291	369	183	.50
18	43	284	369	190	.52
19	41	298	376	185	.49
20	43	284	362	182	.50
21	43	277	355	175	.49
22	42.5	291	369	186	.50
23	41.5	294	376	179	.48
24	42	284	362	176	.49
Avg	42	287	364	182	.5
Adrenalectomized Animals.					
1	43	263	348	151	.44
2	42	263	355	136	.38
3	44	242	340	139	.41
4	44	248	355	114	.32
5	42	254	340	136	.40
6	44	254	362	118	.33
7	45	248	362	111	.31
8	44	254	355	125	.35
9	45	270	369	149	.42
10	43	263	355	142	.40
Avg	43.6	256	354	134	.38

values and from the hematocrit reading. This indirect method was used because it proved difficult to measure pure red cells accurately and because direct determinations showed that there is no significant difference between the calculated and the directly determined values. The determinations were performed with the Rusznyak¹⁸ micro-method which in our experience gives results which check well with those obtained by the Van Slyke method. Table I summarizes our results.

As the table indicates, there is a relatively slight decrease in plasma chlorides, a somewhat more marked decrease in whole blood chlorides and a very pronounced decrease in the red cell chloride concentration. As a result of this, the index $Cl_c : Cl_{P_1}$ (that is, the chloride concentration of the cells divided by the chloride concentration of the plasma) falls considerably. In 7 of 10 animals in the adrenalectomized group the plasma chloride concentration was within the limits of normal variation while in No. 11 among the normals, for instance, the plasma chloride concentration was below the average of the adrenalectomized group. This may explain why many of the authors who based their conclusions merely on plasma chloride determinations obtained inconclusive results. It will be seen that the red cell chloride concentration in all adrenalectomized animals is considerably lower than it is even in the lowest of the normal figures. Similarly the $Cl_c : Cl_{P_1}$ index in all the adrenalectomized animals is lower than the lowest value in the normal group.

Conclusions. Experiments on adrenalectomized rats indicate that the red cell chloride concentration decreases much more markedly during adrenal insufficiency than does the plasma or whole blood chloride content. In cases of slight hypochloremia caused by moderate adrenal insufficiency, the direct or indirect determination of the red cell chloride concentration or of the index $Cl_c : Cl_{P_1}$ is a more sensitive index of a change in chloride metabolism than the more commonly determined plasma or whole blood chloride concentration.

¹⁸ Rusznyak, S., *Biochem. Z.*, 1920, **114**, 23.

The Effect of Sesame Oil ("T-Factor") on the Platelet Count.*

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Schiff and Hirschberger^{1, 2} have reported that sesame oil, which they call the "T-Factor", administered orally or intramuscularly has the specific effect of raising the platelet levels of normal children or young rats. Balereau³ could not repeat these results in 6 normal children or in one child with thrombocytopenic purpura.

In an effort to determine the effect of sesame oil on the thrombocyte level, the platelet counts were determined thrice weekly on 6 adult rats for a period of 50 days. Table I gives a summary of these results. Rats Nos. 1 and 2 were kept as controls. Rats 3 and 4 were given 0.1 cc of sesame oil orally for 17 days and rats 5 and 6 were given a similar dosage for a period of 32 days. All of the animals were kept on standard meal mixture diets and maintained their weights throughout the period of observation. Red cell counts and hemoglobin determinations were done at the same time that the platelets were counted. The solution described by Rees and Ecker was used for the platelet counts. There were no significant changes in the platelet levels of the animals. The hemoglobin determinations and the red cell counts did not change significantly during the period of observation.

TABLE I.
The Effect of Sesame Oil ("T-factor") on the Platelet Counts of 6 Adult Rats.
Platelet Counts in Thousands.

Day of Exp.	Rat No. 1	No. 2	No. 3	No. 4	No. 5	No. 6
1	796	743	749	702	756	769
17	771	725	755	756	769*	762*
33	791	729	779*	743*	782	786
50	771	743	769	741	764	768

* Administration of sesame oil begun.

* This paper forms a part of the study on the physiology and chemistry of blood coagulation which is conducted by Dr. Erwin Chargaff and associates in the Department of Biological Chemistry of this college. This work has been aided by a grant from the John and Mary Markle Foundation.

†It is a pleasure to acknowledge the technical assistance of Miss Hildegard Menzel in this work.

1 Schiff, E., *Jahr. f. Kinderh.*, 1937, **149**, 81.

2 Schiff, E., and Hirschberger, C., *Am. J. Dis. Child.*, 1937, **53**, 32.

3 Balereau, K., *Monatschr. f. Kinderh.*, 1937, **69**, 389.

One adult patient, aged 39 years, with a typical clinical picture of thrombocytopenic purpura was given 15 cc of sesame oil daily for 17 days. At entry to the hospital the platelet count was 18,000 and after treatment, 28,000. This apparent rise was within the limits of error of the method used and was not thought to be significant.

One infant, aged 19 mos, also having thrombocytopenic purpura was given 30 drops of sesame oil orally for 11 days and during this period received 2 small transfusions of blood. The platelet count rose from 12,000 to 74,000. A rise of this magnitude in infants is not unusual in untreated purpura.

In neither the rats nor patients treated with sesame oil did the platelets rise to levels of near or over 1,000,000 as described by Schiff and coworkers.

Summary. Oral administration of sesame oil had no effect on the platelet levels of 6 adult rats or 2 patients with thrombocytopenic purpura.

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Effect of Thyroid Feeding on Androgen Excretion Following Testosterone and Testosterone Propionate Injections in Rabbits.

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We have been unable to detect any comb growth-promoting substance in the urine of untreated normal adult male rabbits or in the feces of male rabbits even when they were given 10 mg of testosterone or testosterone propionate daily for 4 or 5 days. On the other hand, the above mentioned doses of these androgens regularly lead to the excretion of considerable amounts of androgen in the urine. This result is confirmatory of the observations of McCullagh, *et al.*,¹ Hoskins, *et al.*,² Dorfman and Hamilton,³ and many others, and is at variance with the observations of Kochakian.⁴

In these experiments 5 mg of testosterone or testosterone pro-

¹ McCullagh, E. P., Rumsey, J. M., and Cuyler, W. K., *Proc. Cent. Soc. Clin. Res.*, 1938, **11**, 18.

² Hoskins, W. H., Coffman, J. R., Koch, F. C., and Kenyon, A. T., *Endocrinology*, 1939, **24**, 702.

³ Dorfman, R. I., and Hamilton, J. B., *J. Clin. Invest.*, 1939, **18**, 67.

⁴ Kochakian, C. D., *Endocrinology*, 1939, **24**, 331.

TABLE I.

Rabbit No.	Days of urine collection	Androgen used (daily dose 10 mg)	No. days given	Desic. thyr. No. (daily dose mg)	No. days given	Thyroid status	Testes status	Comb growth 6 days H + L mm	Total androgen excretion as androstosterone mg
1702	4	Testosterone propionate	4		Intact	Intact		8%	2.82
1702	5	"	5		Thyroidectomy		Cryptorchid	7%	2.36
1702	5	"	5	100		"	"	11%	4.00
1703	4	"	4		Intact	Intact		9	2.91
1703	5	"	5		Thyroidectomy		"	7%	2.36
1703	5	"	5	100		"	"	12	4.36
1430	4	"	4		"	"	"	51%	1.55
1430	4	"	4	100		"	"	9%	3.27
1599	4	"	4		"	Gonadectomy		61%	1.91
1599	4	"	4	100		"	"	7%	2.36
1703	4	Testosterone	4		"	Cryptorchid		9%	3.26
1703	4	"	4		"	"	"	101%	3.55
1703	5	"	5		"	"	"	11%	4.18
1702	5	"	5	100		"	"	121%	4.55

pionate* in 1 cc of sesame oil were injected into the abdominal wall twice daily for 4 or 5 days with or without the daily oral administration of 100 mg of desiccated thyroid for similar periods. Desiccated thyroid administration was begun 1 day before the first dose of androgen. The urine was collected under benzene and extracted according to the method of Dingemanse and Laqueur.⁵ The extracts were assayed by Fussgänger's⁶ modification of the capon comb test. The essential data of the experiments are given in Table I.

It will be seen that 2 intact rabbits receiving 40 mg of testosterone propionate excreted slightly more urinary androgen than the same rabbits after thyroidectomy and the intraabdominal suspension of their testes, although 50 mg were given. When 50 mg of testosterone propionate were injected and 500 mg of desiccated thyroid given there was nearly twice as much androgen excreted in 3 of the 4 rabbits. The rabbit (1599) with only a slight increase in androgen excretion following desiccated thyroid administration had been gonadectomized. When testosterone was given to the same rabbits there was no difference in the androgen excretion whether or not desiccated thyroid was administered.

These experiments explain from another angle the greater physiological activity of testosterone propionate and suggest that there may be little or no difference between the action of testosterone and testosterone propionate if the latter is given with desiccated thyroid. They further indicate that the more prolonged action of testosterone propionate is due to delayed hydrolysis rather than differences in the rate of absorption and that the thyroid hormone hastens the splitting of the ester after absorption. One could also infer from these experiments that the esters of testosterone would be physiologically more effective after subtotal thyroidectomy, and this is indicated in their exophthalmos promoting effect.⁷

* We are indebted to Ciba Products, Inc., for the testosterone and testosterone propionate (Perandren).

⁵ Dingemanse, E., and Laqueur, E., *Biochem. J.*, 1937, **31**, 500.

⁶ Fussgänger, R., *Med. u. Chem. Forschungstätten*, 1934, **2**, 194.

⁷ Marine, D., and Rosen, S. H., *Proc. Soc. Exp. Biol. and Med.*, 1938, **38**, 353.

Inhibiting Effect of Thyroidectomy on Adrenal Cortex Hypertrophy Following Injections of Anterior Pituitary Extract.

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The rôle of the thyroid gland in the hypertrophy of the adrenal cortex following administration of anterior pituitary substance has been a subject of considerable investigation with reports that are conflicting. Some experimenters^{1, 2, 3} have concluded that this hypertrophy of the adrenal cortex is mediated largely or entirely through the thyroid gland. Others^{4, 5, 6, 7, 8} have found that the presence of the thyroid gland is not necessary for this reaction. In view of these conflicting reports we carried out similar experiments on the guinea pig.

Twenty-three young guinea pigs of both sexes were used in 2 series of experiments. In the first there were 12 animals (6 males and 6 females) ranging in age at the beginning of injections from 28 to 34 days, and in weight from 213 to 364 g. Four were thyroidectomized, 3 gonadectomized, 3 thyroidectomized and gonadectomized, and 2 unoperated. Gonadectomy was performed 11 days before, and thyroidectomy 7 days after, beginning injections. All animals received daily intraperitoneal injections of anterior pituitary extract for 48 days, and were sacrificed 2 to 5 days after the last injection. In the second experiment there were 11 guinea pigs (6 males and 5 females) ranging in age at the beginning of injections from 32 to 38 days, and in weight from 251 to 380 g. Litter-mate pairs of the same sex were chosen, one of each pair being thyroidectomized 8 days before beginning treatment. All animals received daily intraperitoneal injections of anterior pituitary extract, and were sacrificed in pairs after 7, 8, 9, 19 and 20 days. The extract used was a 0.5% acetic acid extract of dried beef anterior pituitary prepared, with slight modifications, according to the

¹ Loeser, A., *Arch. f. exp. Path. u. Pharm.*, 1933, **173**, 62.

² Emery, F. E., and Winter, C. A., *Anat. Rec.*, 1934, **60**, 381.

³ McQueen-Williams, M., *PROC. SOC. EXP. BIOL. AND MED.*, 1934, **32**, 296.

⁴ Houssay, B. A., Biasotti, A., Mazzoco, P., and Sammartino, R., *Comp. Rend. Soc. Biol.*, 1933, **114**, 737.

⁵ Atwell, W. J., *Am. J. Physiol.*, 1937, **118**, 452.

⁶ Jores, A., and Boecker, W., *Zeitsch. f. d. ges. exp. Med.*, 1936-37, **100**, 332.

⁷ Moon, H. D., *PROC. SOC. EXP. BIOL. AND MED.*, 1937, **35**, 649.

⁸ Collip, J. B., *Lancet*, 1933, **2**, 347.

TABLE I.

G.P. No.	Sex	Total dosage ant. pit. extr. dried gland equivalent, g	Duration of exp., days	Body wt at end, g	Gain or loss in body wt, g	Adrenal wt, g	Thyroid wt, g	Gonads wt, g	Prostate and seminal vesicles or uterus wt, g
478	F	3.9	7	314	+ 20	.132		.073	1.026
479	F	3.9	7	245	— 6	.158		.064	.785
484	M	4.5	8	320	+ 24	.117	Frag. $3.5 \times 1.5 \times 1$ mm	.734	1.413
485	M	4.5	8	278	+ 13	.113		.585	.942
480	M	5.5	9	314	+ 34	.161	2 Frag. 1.5 mm	1.2	2.0
481	M	5.5	9	312	— 21	.143		2.0	1.6
474	F	8.4	19	341	+ 9	.183	Frag. 1 mm	.083	.764
475	F	8.4	19	348	+ 2	.235		.089	.408
482	M	8.6	20	363	+ 43	.174		2.1	4.2
483	M	8.6	20	352	+ 5	.201		1.018	4.1
477	F	8.6	20	388	— 19	.238		.080	.429
469	F	20.9	50	373	+ 84	.174	Frag. 1 mm	.075	.742
468	F	20.9	53	407	+ 88	.265	Frag. $5 \times 3 \times 1.5$ mm	.080	1.520
467	F	20.9	52	376	— 43	.253		.047	.369
464	M	20.9	51	404	+ 143	.165		2.703	3.5
465	M	20.9	52	442	+ 141	.175	Frag. microscopic	1.406	6.4
466	M	20.9	50	468	— 22	.302		2.0	3.9
459	M	3.5	7	232	— 27	.117			.565
458	M	20.9	50	420	+ 100	.223			.443
460	M	20.9	51	331	+ 114	.163			.325
463	F	20.9	52	371	+ 103	.250		.089	.223
462	F	20.9	50	265	+ 52	.184			.392
461	F	20.9	53	401	+ 152	.191			.268

The first 10 guinea pigs are grouped as littermate pairs of the same sex, one of each pair being thyroidectomized. The last 12 pigs are grouped according to sex and operative procedure.

method of Loeb, and concentrated so that one cc was equivalent to 0.1 to 0.5 g of dried gland. The daily dose was 1 or 2 cc.

The principal data of the experiments are given in Table I.

The adrenal glands of completely thyroidectomized guinea pigs and of those in which only minute fragments of thyroid were found were considerably and consistently smaller than the adrenals of the controls. This was true of both sexes. The greatest differences in adrenal weights of thyroidectomized and control animals were observed in the pigs injected for the longest time. In 3 instances where large thyroid fragments were found in thyroidectomized guinea pigs the weights of their adrenals were similar to those of the controls. The same observation was made by Emery and Winter.²

Gonadectomy had little or no effect on the hypertrophy of the adrenals caused by injection of anterior pituitary extract. This confirms reports in the literature.^{2, 4, 9} Thyroidectomy and gonadectomy had essentially the same effect as thyroidectomy alone.

Thyroidectomy, or subtotal thyroidectomy, tended to increase the weight response of the gonads and accessory reproductive organs to injections of anterior pituitary extract. This increased response was consistent and marked in the case of the uterus, but was not so consistent in the case of the prostate and seminal vesicles, or the gonads. The exceptions could not be correlated with other findings. Also, larger accessory reproductive organs in the thyroidectomized animals were not always associated with larger gonads. Most evidence in the literature indicates that thyroidectomy increases the response of the gonads and accessory reproductive organs to anterior pituitary stimulation.^{10, 11, 12}

The thyroid gland clearly plays an important part in the hypertrophy of the adrenal cortex induced by anterior pituitary extract. The frequently observed hypertrophy of the adrenal cortex following the administration to animals of desiccated thyroid or thyroxine emphasizes this relationship of thyroid to adrenals.¹³ Other experimental evidence indicates the reciprocal nature of the thyroid-adrenal relationship. A rise in respiratory metabolism following sublethal injury of the adrenals was reported by Marine and Baumann¹⁴ in rabbits, and was confirmed by Davis and Hastings¹⁵ in

⁹ Anselmino, K. J., Hoffmann, F., and Herold, L., *Klin. Wschr.*, 1933, **2**, 1944.

¹⁰ Schockaert, J. A., *Comp. Rend. Soc. Biol.*, 1931, **108**, 431.

¹¹ Leonard, S. L., *Proc. Soc. Exp. Biol. and Med.*, 1936, **34**, 599.

¹² Fluhmann, C. F., *Am. J. Physiol.*, 1934, **108**, 498.

¹³ Hoskins, R. G., *J. Am. Med. Assn.*, 1910, **55**, 1724.

¹⁴ Marine, D., and Baumann, E., *Am. J. Physiol.*, 1921, **57**, 135.

¹⁵ Davis, J. E., and Hastings, A. B., *Am. J. Physiol.*, 1933, **105**, 110.

mice. Previous thyroidectomy, when complete, prevented this rise in metabolism in rabbits.¹⁶ On the other hand, feeding a glycerol emulsion of fresh beef adrenal cortex to rabbits lowered the respiratory metabolism.¹⁷ Oehme¹⁸ obtained a similar lowering of metabolism with an adrenal cortex extract in thyroxine treated guinea pigs. Also significant are the observations that feeding a residue of whole beef adrenal to dogs caused a marked increase in iodine content of the thyroid gland,¹⁹ and that administering corticotropic hormone to dogs definitely lowered the blood iodine and caused involution of the thyroid gland to the colloid state.²⁰

10775 P

Electrical Anesthesia in Rats.

M. L. SILVER. (Introduced by R. W. Gerard.)

From the Department of Physiology, University of Chicago.

Electrical anesthesia in mammals, produced by means of an interrupted direct current, is reported by Leduc,¹ Robinovitch,² von Neergard,³ and Ivy and Barry,⁴ and denied by Sack and Koch.⁵ Von Haareveld and Kok⁶ obtained narcosis in the dog with a sinusoidal current and Scheminzky and others⁷ in the frog with a constant one. Conflicting results may have prevented the use of electro-narcosis in the clinic, yet it seems to offer much promise. This is a preliminary report of the successful production of anesthesia in rats by a constant direct current.

Large dry cells (145 volts), a graphite rheostat, a milliammeter, and a reversing switch are connected in series with a rat through

¹⁶ Marine, D., and Baumann, E., *Am. J. Physiol.*, 1922, **59**, 353.

¹⁷ Marine, D., and Baumann, E., *Am. J. Physiol.*, 1925, **72**, 248.

¹⁸ Oehme, C., *Klin. Wschr.*, 1936, **1**, 512.

¹⁹ Black, E. M., Hupper, M., and Rogers, J., *Am. J. Physiol.*, 1922, **59**, 222.

²⁰ Reiss, M., and Peter, F., *Zeitsch. ges. exp. Med.*, 1938, **104**, 49.

¹ Leduc, S., *Arch. d'Elect. Med.*, 1902, **10**, 769.

² Robinovitch, L. G., *Sommeil électrique, épilepsie électrique et électrocution*, Thèse, Paris, 1906.

³ von Neergard, K., *Arch. für Klinische Chirurgie*, 1923, **122**, 100.

⁴ Ivy, A. C., and Barry, F. S., *Am. J. Phys.*, 1932, **99**, 298.

⁵ Sack, G., and Koch, H., *Z. f. d. g. Exp. Med.*, 1933, **90**, 349.

⁶ von Haareveld, A., and Kok, D. J., *Arch. neêrl. Phys.*, 1934, **19**, 24.

⁷ Scheminzky, F., Hochstädt, O., and Adler, P., *Pflüger's Arch.*, 1936, **237**, 284.

non-polarizable, zinc-zinc sulfate electrodes. The cathode is firmly pressed against the roof of the mouth and the anode is inserted about 1 cm into the rectum. The animal lies with his legs dependent through holes in a platform.

The rat is usually anesthetized with ether prior to the application of current, to avoid struggling against confinement. Control animals without current, emerge from this anesthesia in 5 minutes (never more than 10 minutes). The experimental animals are subjected to a current of 10 mA (rheostat at 11,500 ohms) gradually increased from zero during a period of 3 minutes. As long as this current flows, the animal remains quiescent and gives no response to severe noxious stimuli, such as cutting, burning, or powerful tetanizing shocks. (Tests have been continued up to 4 hours. After a half-hour the anesthetizing current can be reduced to 8 mA.) To terminate anesthesia, the current is decreased to 4 mA during a minute, its direction reversed for 10 seconds, and then discontinued. The animal regains normal reflex thresholds within 5 minutes; fully normal behavior returns within 10 minutes.

Repeated periods of electroanesthesia (10 hours total anesthesia) have led to no detectable physiological defects or neurohistological changes.

With the cathode on the shaved skin of the shoulder region instead of in the mouth, the head and forelimbs are largely outside the current path and are spared its narcotic action. Such rats respond normally to stimulation of the fore parts, not at all to stimulation of the hind parts.

Studies on the site and mechanism of action of the current are being continued. Reflex block is definitely in the central nervous system, and not in peripheral structures.

Effect of Anoxemia upon Electrocardiogram of Cats after Coronary Ligation.

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Columbia University.*

Ligation of the left branch of the left anterior descending coronary artery in cats results in typical "coronary" electrocardiographic records. These abnormal tracings frequently disappear in 2 to 3 weeks despite the persistence of grossly demonstrable infarcts at autopsy. Following the suggestion of Levy, Bruenn, and Russell¹ that the electrocardiographic changes which anoxemia induces in patients with coronary disease, may be of diagnostic value, these cats were subjected to mild anoxemia. When normal cats, anesthetized with pentobarbital, were made to breathe 10% oxygen in nitrogen for 20 minutes, the electrocardiographic changes induced were slight, and unlike those following coronary ligation.

Immediately after coronary ligation, the electrocardiograms of 7 of the 8 cats studied showed deviation of the RS-T segment in 19 of 21 leads. The electrocardiogram of the eighth cat was normal, but subsequently showed RS-T segment deviation. The T-waves were generally increased in amplitude. At this time, the induction of mild anoxemia exaggerated the RS-T deviations in 13 of the 19 leads, and elicited RS-T deviations in the electrocardiogram of the one cat in which they were lacking postoperatively. T-wave changes were slight and variable. The induction of the anoxemia was repeated at weekly intervals thereafter and continued to exaggerate the RS-T deviations, or to cause their reappearance.

Meanwhile, the RS-T segments of the electrocardiograms of the non-anoxemic cats approached the iso-electric line, and the electrocardiograms of 6 of the 8 cats returned to normal within from 12 to 29 days. One of the 2 cats whose electrocardiograms continued to show "coronary" changes was sacrificed 14 days postoperatively because of impending exitus from "snuffles." The other continued to show electrocardiographic changes until it was sacrificed, 50 days postoperatively. When the electrocardiograms had returned to normal, the induction of anoxemia resulted in the

¹ Levy, R. L., Bruenn, H. G., and Russell, N. G., Jr., *Am. J. Med. Sci.*, 1939, **197**, 241.

reappearance of "coronary" complexes in every case. Thirteen of 18 leads showed deviation of the RS-T complex.

After experimental coronary ligation, the resulting infarct is composed of a central area of ischemic tissue, surrounded by a relatively narrow periphery of edematous, anemic, but still viable myocardium. Since it was shown that the electrocardiograms of such cats frequently resume normal contours in the presence of a fibrotic area, it is suggested that the electrocardiographic changes which had been present were due to local myocardial anoxia and not to the mere presence of a mass of scar tissue. If it is assumed that the electrocardiograms return to normal when the local anoxia has fallen below a given threshold value, the induction of mild anoxemia would cause the reappearance of "coronary" changes. By raising the level of local anoxia above the threshold, its effects upon the electrocardiogram would become manifest. This was actually accomplished.

10777

Avitaminosis B₁ and Pigeon Brain Potentials.

E. TOKAJI AND R. W. GERARD.

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Since vitamin B₁ lack disturbs the normal carbohydrate metabolism of brain, it was desirable to follow its influence on brain potentials. Pigeons were standardized on a stock diet for 30 days, then on a diet of polished rice and salt.¹ Although deficient in other nutritional elements, only vitamin B₁ lack could play a rôle within the time limits, for typical beri-beri symptoms (opisthotonus) developed in 30-40 days. A small portion of the calvarium over the occipital portion of one cerebral hemisphere was removed under nembutal (30 mg/kg, sufficient to relax the rigid muscles) and the exposed brain kept moist and warm with Ringer. Potentials were led off (different electrode on exposed brain; indifferent on bone over opposite hemisphere) with Ag-AgCl wick electrodes,² amplified and recorded with a crystograph. When desired, 2-5 mg of crystalline vitamin B₁ were injected intramuscularly, administered

¹ Kinnersley, H. W., Peters, R. A., and Reader, V., *Biochem. J.*, 1928, **22**, 276.

² Libet, B., and Gerard, R. W., *J. Neurophysiol.*, 1939, **2**, 153.

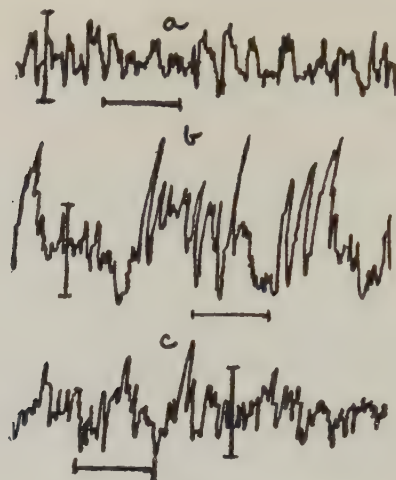


FIG. 1.

a. Normal brain.

b. Avitaminous brain.

c. Same brain 2 hours after intramuscular injection of 2.5 mg thiamine.

Time—1 sec. Amplitude—100 μ V.

orally, or both. At the conclusion of the experiment, the scalp was sewn together. Despite the absence of aseptic technique no sepsis resulted and repeated records could be obtained from one bird.

Normal brain potentials (Fig. 1a). A predominant 10-25 per sec. rhythm of 10-30 μ V is usually superimposed on one of 2-5 per sec. and 80-100 μ V. A 35-50 per sec. rhythm of less than 10 μ V is often superimposed on both of the preceding ones. Occasional "spike-like" potentials of 100 μ V and more interrupt the slower waves. The several frequencies are not very regular, individually or in their sequence.

Potentials from the avitaminous brain (Fig. 1b) are more regular than in the normal. The fastest rhythm is unchanged or decreased, the slower ones are increased in amplitude by as much as 100%, and more and larger spikes appear.

Administration of vitamin B₁ does not influence potentials of the normal brain. In avitaminous pigeons, deficiency symptoms disappear within 2 hours of its injection, paralleled by a reversion of potentials toward normal (Fig. 1c). Brain potential records were controlled for muscle, eye and other stray potentials.

Although a definite change in brain potentials can be observed when the normal pigeon becomes avitaminous and this is reversed by thiamine administration, it is too variable in detail to permit

quantitative analysis. O'Brien and Peters³ showed that oxygen consumption is moderately reduced in the avitaminous brain coupled with impairment of carbohydrate metabolism. This parallelism to insulin shock is further shown by the changes in brain potentials recorded here and, under insulin, by Dubner and Gerard,⁴ Himwich *et al.*,⁵ and others.

10778 P

Further Experience With the Use of Thrombin as a Hemostatic Agent.*

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H. P. SMITH.

From the Department of Pathology, State University of Iowa, Iowa City.

We have recently reported animal experiments¹ on the use of purified thrombin² as a hemostatic agent. We are now able to obtain thrombin solutions free of bacteria, and with minor loss of activity, by use of fritted glass filters.³ We wish to report additional toxicity experiments; also preliminary experience with the use of thrombin in a small group of human cases.

Toxicity Experiments. As pointed out previously,¹ thrombin, applied to operative surfaces, is non-toxic. Numerous other toxicity experiments with thrombin have since been performed, and a small group of these is presented below.

(1) Intramuscular administration. An injection of 6000 units into the thigh muscles of an adult rat produced no local thrombosis, and no clinical evidence of toxicity. Blood samples drawn several

³ O'Brien, J. R., and Peters, R. A., *J. Physiol.*, 1935, **85**, 454.

⁴ Dubner, H., and Gerard, R. W., *J. Neurophysiol.*, 1939, **2**, 142.

⁵ Himwich, H. E., Hadidian, Z., Fazekas, J. F., and Hoaglund, H., *Am. J. Physiol.*, 1939, **125**, 578.

* Aided by a grant from the John and Mary R. Markle Foundation. Funds for two research assistants were supplied by the Graduate College, State University of Iowa.

¹ Seegers, W. H., Warner, E. D., Brinkhous, K. M., and Smith, H. P., *Science*, 1939, **89**, 86.

² Seegers, W. H., Brinkhous, K. M., Smith, H. P., and Warner, E. D., *J. Biol. Chem.*, 1938, **126**, 91.

³ Morton, Harry E., and Czarnetzky, E. J., *J. Bact.*, 1937, **34**, 461.

hours later showed no alteration in the plasma fibrinogen or prothrombin levels.

(2) Intraperitoneal administration. An injection of 6000 units of thrombin (4 cc) caused some uneasiness in a 300 g rat, possibly because the solution was hypotonic. The blood, 6 hours later, showed almost complete disappearance of the fibrinogen, and a 50% reduction in the plasma prothrombin. Intraperitoneal injection of 1500 units into a 200 g rat produced a very slight reduction in the plasma fibrinogen; 700 units had no effect. A proportionate dose in man would be at least 100 cc of concentrated thrombin solution.

(3) Intravenous administration. Rapid injection of 0.5 cc thrombin (250 units) into the jugular vein of a 300 g rat resulted in death in 60 seconds. Thrombi were found in the right ventricle and in the pulmonary artery. Injection of 100 units into a 285 g rat produced no obvious disturbance.

Thrombin, intravenously, is highly dangerous, yet the rat will tolerate larger amounts than one might expect, for 100 units of thrombin will clot 50 cc of oxalated blood *in vitro* in 16 seconds.

Hemostasis in Man. Because of difficulties in preparing large amounts of thrombin on a laboratory scale, the accumulation of experience in human cases is necessarily slow. We have now used thrombin in 21 human cases, 4 of which are tabulated to show its hemostatic possibilities.

Effective hemostasis depends upon formation of clots which seal the ends of tiny vessels. Large vessels must be ligated. If oozing is slow, prompt hemostasis can usually be obtained by applying thrombin with an atomizer. If the bleeding is brisk, however, the clot which forms often fails to adhere to the tissue, thus permitting

TABLE I.

Case No.	Bleeding Site	Thrombin units/cc	Method of application	Results
1	Operative, radical mastoidectomy	1500	Spray atomizer	Filmy clots formed in few seconds. Bleeding controlled with 1-3 applications.
2	Skin graft, donor areas, excessive oozing	1500	Spray atomizer	Hemorrhage effectively checked. Some areas required second application.
3	Two tooth sockets, bleeding 24 hr (hemophilia)	2500	Jet from hypodermic needle	Hemorrhage promptly checked.
4	Tiny stab wound, ear ("pseudo-hemophilia")	1000	Dropped into wound	Hemorrhage promptly checked.

continued oozing underneath the clot. In fact, the clot on the surface acts as a barrier between the fluid blood and the thrombin being applied. In such instances more satisfactory results are often obtained by applying the thrombin in a fine jet under pressure, thus forcing the thrombin into contact with the tissues. Further studies with this and other methods of application are being made.

10779

Inactivation of Prothrombin by Purified Thrombin Solutions.*

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From the Department of Pathology, State University of Iowa, Iowa City.

In some recent experiments on the conversion of prothrombin into thrombin, we discovered a new reaction in which prothrombin is inactivated. It is our present purpose to present data regarding this inactivation, and to discuss the probable nature of the reaction.

Methods. Thromboplastin: Mix 100 g fresh ground beef lung with 100 cc saline. Allow to stand, with occasional stirring, for 48 hours at 5°. Centrifugalize and dilute the fluid obtained with an equal volume of saline. Any prothrombin present is removed by adsorption (one-sixth volume of $\text{Mg}(\text{OH})_2$ suspension¹) followed by centrifugalization. To 100 cc of the clear adsorbed solution add 100 cc $(\text{NH}_4)_2\text{SO}_4$ solution (saturated at 5°). Centrifugalize and dissolve the precipitate, containing thromboplastin, in 100 cc saline. Repeat the precipitation, and dissolve the final precipitate in 15 cc saline. Dialyze against saline until free of $(\text{NH}_4)_2\text{SO}_4$. The entire procedure is carried out in the cold room (5°). The product used in the present series of experiments contained 26 mg organic solids per cc.

To prepare an isotonic buffer solution (pH 7.25) which does not interfere with the action of calcium ion, dissolve 1.72 g imidazole (Eastman Kodak) in 90 cc of 0.1 N HCl, and dilute to 100 cc with H_2O .

* Aided by a grant from the John and Mary R. Markle Foundation. Funds for two research assistants were supplied by the Graduate College, State University of Iowa.

¹ Smith, H. P., Warner, E. D., and Brinkhous, K. M., *J. Exp. Med.*, 1937, **66**, 801.

The titration of prothrombin was carried out by the 2-stage method of Warner, Brinkhous and Smith.^{1, 2}

Experimental. In Fig. 1 are shown experiments in which prothrombin was treated with thromboplastin and calcium in measured amounts. A solution was first prepared containing 0.9% NaCl and 0.15% $\text{Ca}(\text{NO}_3)_2$. To 14 cc of this was added 1.0 cc of the imidazole buffer solution. Purified prothrombin³ was added in solid form in such amounts that each cc contained 2250 units prothrombin (approximately 25 mg). The thromboplastin solution, above described, was then added in measured amounts. By this addition the prothrombin-calcium-buffer mixture was diluted somewhat. The data given in Fig. 1 contain a correction for this dilution.

When 1250 gamma (1.25 mg) thromboplastin were added, the conversion of prothrombin into thrombin was completed within 6 minutes (upper curve), and thereafter the thrombin titer remained constant throughout the 11-hour period of observation, showing that the clotting agents had been prepared free of antithrombin. Data not given in the figure showed that this quantity of thromboplastin was a great excess, for complete conversion of 2250 units of prothrombin could be obtained by use of as little as 200 gamma of this thromboplastin. The additional thromboplastin does not increase the thrombin titer, but merely increases the speed of the reaction.

With the use of a much smaller amount of thromboplastin (25 gamma), 450 units of thrombin were promptly formed, and for several hours thereafter the thrombin titer remained at this same level. This sharply limited capacity of thromboplastin to produce thrombin is proof that thromboplastin is consumed in the reaction. This is an important fact which will be discussed in a later paper.

The above mixture, containing 450 units of thrombin, was allowed to stand for a period of 350 minutes. During this period the prothrombin suffered partial inactivation, for one could no longer raise the thrombin titer to the 2250 unit level by adding thromboplastin in excess. Furthermore, the thrombin which did form under these conditions made its appearance very slowly. Controls not given in Fig. 1, showed that this inactivation of prothrombin had occurred gradually throughout the first 4 hours of the experiment. During the first half hour almost all of the prothrombin

² Warner, E. D., Brinkhous, K. M., and Smith, H. P., *Am. J. Physiol.*, 1936, **114**, 667.

³ Seegers, W. H., Smith, H. P., Warner, E. D., and Brinkhous, K. M., *J. Biol. Chem.*, 1938, **123**, 751.

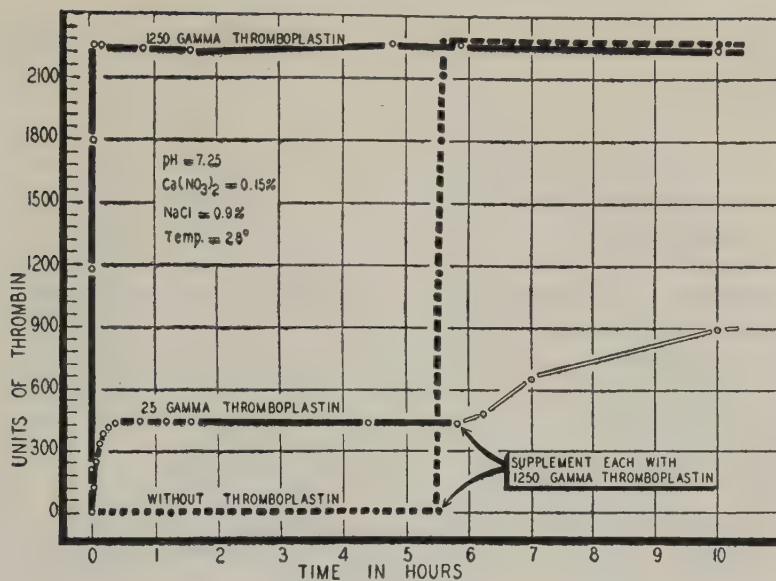


FIG. 1.
Thrombin formation and prothrombin destruction.

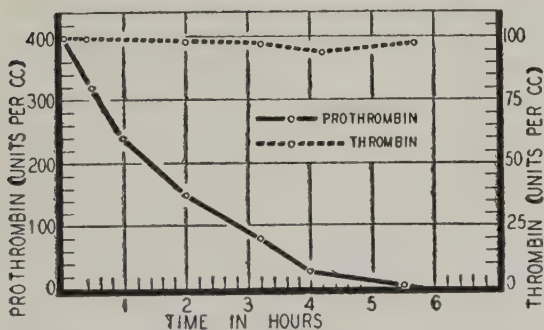


FIG. 2.
Prothrombin inactivation by means of a purified thrombin preparation. The mixture contained 400 units of purified prothrombin and 100 units of purified thrombin. It also contained the imidazole buffer solution (1 volume in 15) and NaCl to the extent of 0.9% and potassium oxalate to the extent of 0.045%. Temperature of experiment 28°.

originally present could be converted into thrombin by the addition of large amounts of thromboplastin. During the following 3.5 hours the excess prothrombin gradually lost its ability to form thrombin, giving finally the weak response described above.

This alteration of prothrombin is clearly not a change which occurs spontaneously in prothrombin, for we have repeatedly found that purified solutions of this material retain their activity for many hours at room temperature. Nor is the inactivation due

to the action of thromboplastin, for when prothrombin and thromboplastin are incubated in the absence of calcium the prothrombin retains its activity for long periods of time. Furthermore, the lower curve of Fig. 1 shows that calcium does not inactivate prothrombin. The two substances were left in contact for over 5 hours, after which thromboplastin was added. A total of 2250 units of thrombin promptly formed, showing that the prothrombin had retained its activity in full.

Since the destruction of prothrombin is due neither to calcium nor to thromboplastin, it would appear that thrombin itself may be the agent responsible. Unless this is true, one must assume that the destructive agent is one which appears simultaneously with the formation of thrombin, and, like thrombin, requires both calcium and thromboplastin for its production.

Fig. 2 shows an experiment which gives further evidence that thrombin is the substance which causes the inactivation of prothrombin. Purified solutions of prothrombin and thrombin were mixed and allowed to stand at room temperature for a period of 6 hours. At intervals the prothrombin titer was determined as the increment of thrombin produced in 6 minutes by adding calcium in optimal amounts (0.15% $\text{Ca}(\text{NO}_3)_2$) and thromboplastin in excess. As Fig. 2 shows, a marked fall in the prothrombin titer began almost at once, and at the end of 6 hours very little prothrombin activity remained. The thrombin used in this experiment was purified by a technic previously described⁴ and the excess thromboplastin used in its formation was largely removed in the process. In the present experiment (Fig. 2) calcium ion was eliminated by including potassium oxalate in the mixture. It is thus clear that the agent which destroys prothrombin is present in the thrombin preparation itself. Since the thrombin was purified by repeated precipitation, it seems likely that the destructive agent is thrombin itself and not some impurity.

We have conducted a few preliminary experiments on heat inactivation of purified thrombin solutions. A temperature of 60° for 30 minutes causes considerable loss in both the clotting power and in the ability to inactivate prothrombin. The loss in each respect cannot yet be said to be quantitatively identical, but they are clearly of the same order of magnitude.

The evidence that thrombin causes inactivation of its own precursor, prothrombin, introduces an apparent anomaly. This con-

⁴ Seegers, W. H., Brinkhous, K. M., Smith, H. P., and Warner, E. D., *J. Biol. Chem.*, 1938, **126**, 91.

clusion, however, is somewhat analogous to the recent conclusion of Kunitz⁵ that trypsin can convert its precursor, trypsinogen into an inert protein.

It is of interest that in the experiment of Fig. 2 very little thrombic activity was lost during the 6-hour period of incubation. This might be taken to indicate that thrombin acts enzymatically to inactivate prothrombin, but further work is needed before this question can be answered definitely.

Summary. Evidence is presented to show that solutions of purified thrombin contain a substance which reacts slowly with prothrombin, causing inactivation of the latter. The destructive agent is not present in the calcium, prothrombin, or thromboplastin solutions from which the thrombin is produced. The factor is heat-labile and the evidence indicates that thrombin itself is the factor in question.

⁵ Kunitz, M., *J. Gen. Physiol.*, 1939, **22**, 293.

SECRETARY'S REPORT

April 1, 1938 to April 1, 1939

The members of the Society are requested to note the following:

Election of Officers. The Council, including the Chairman of each Section, nominated the national officers. Three ballots were taken and the results confirmed at the annual meeting in Toronto on April 28. The President appointed three tellers who counted the ballots. The following were elected:

President	J. T. Wearn
Vice-President	C. D. Leake
Secretary-Treasurer	A. J. Goldforb
Councillors	D. W. Bronk
	W. J. Meek
Other members of Council	H. W. Smith (hold over)
	E. A. Doisy " "
	H. S. Gasser (past president)
	Chairman of each Section

It is suggested that hereafter two or more nominations be made for each office.

Annual Meetings of the National Committees. The Board of Editors and the Council have met annually for a number of years. These meetings have been most useful in avoiding misunderstandings, giving a realization of local problems in relation to the needs of the National Society, with resultant better coöperation. This year, the National Membership Committee and the Sectional Secretaries also met at Toronto April 28. It is hoped that these four national committees will meet at the forthcoming annual meetings held during the Federation meetings.

Election to Membership

1. The National Membership Committee is composed of one member from each of the nine largest Sections of the Society.

2. Unanimous approval of applicant by the National Membership Committee constitutes election to membership.

3. When one to 3 vote to reject or defer, application is submitted to entire committee for second ballot.

4. When one to 4 then vote to reject or defer, application is referred to the Council at its annual meeting.

5. When majority vote to reject, this constitutes a rejection, and sponsor is notified.

6. All announcements of election to membership to be made after annual meeting of the Council.

7. Applications from regions where there is no Section are to be referred for endorsement to the nearest Sectional Membership Committee.

Editors. In conformity with the policy of rotation of personnel of the Editorial Board, the following additions and changes are announced: New editors, Doctors S. R. Detwiler (Transplantation), W. S. Tillet (Bacteriology), O. Wintersteiner (Chemistry of hormones); Replacements, F. C. Mann

(Physiology), P. E. Smith (Endocrinology). Due to enlargement of the Board of Editors and the few manuscripts in metabolism, this division is taken over by the editors in physiology.

The Council wishes to give expression of its deep appreciation of the sterling work of Doctors A. B. Luckhardt, G. W. Corner and W. S. McCann as editors of the PROCEEDINGS.

Editorial Policies (clarification or new).

1. In introducing the manuscript of a non-member, the sponsor is assumed to be familiar with the nature of the work, the ability of the investigator and assumes financial responsibility.

2. No distinction is to be made between "clinical" vs. "non-clinical" manuscripts. Selection is based upon adequacy of controls, data, conclusions, etc.

3. The present policy of sending authors the criticisms of the Board of Editors is to be continued. This refers to revision or rejection of manuscripts.

4. To enable prompt publication manuscripts with minor suggestions of the Board of Editors are sent directly to press. Galley proof will state, "Note editorial changes, if any, made to reduce delay in publication."

Instructions to Authors. Please note the changes in "Instructions to Authors" in the PROCEEDINGS.

Costs. Members receive the equivalent of three volumes a year. They pay \$4.00 a year plus 20% of cost for excess space and 50% of cost for cuts. The Treasurer's report on page 667 shows a profit. It is hoped progressively to reduce present low costs to members.

Reprints are limited to 500. When a responsible research institute distributes "collected reprints," more than 500 reprints of an article may be permitted.

Preliminary vs. Complete Manuscripts. For the year ending April 1, 1939 the PROCEEDINGS included 48% preliminary, 52% complete manuscripts.

PAST OFFICERS

<i>Date</i>	<i>President</i>	<i>Vice-President</i>	<i>Secretary</i>	<i>Treasurer</i>
1903-04	S. J. Meltzer	W. H. Park	W. J. Gies	G. N. Calkins
1904-05	S. J. Meltzer	J. Ewing	" "	" "
1905-06	E. B. Wilson	E. K. Dunham	" "	" "
1906-07	S. Flexner	E. K. Dunham	" "	" "
1907-08	S. Flexner	T. H. Morgan	" "	" "
1908-09	F. S. Lee	T. H. Morgan	" "	G. Lusk
1909-10	F. S. Lee	W. J. Gies	E. L. Opie	" "
1910-11	T. H. Morgan	W. J. Gies	" "	" "
1911-12	T. H. Morgan	P. A. Levene	G. B. Wallace	" "
1912-13	J. Ewing	P. A. Levene	" "	C. Norris
1913-14	J. Ewing	C. W. Field	H. C. Jackson	" "
1914-15	G. Lusk	W. J. Gies	" "	J. R. Murlin
1915-16	G. Lusk	G. N. Calkins	H. C. Jackson	" "
1916-17	J. Loeb	W. J. Gies	" "	" "
1917-19	W. J. Gies	J. Auer	" "	" "
1919-21	G. N. Calkins	G. B. Wallace	" "	" "
1921-23	G. B. Wallace	J. W. Jobling	" "	" "
1923-24	H. C. Jackson	J. W. Jobling	V. C. Myers	" "
1924-25	H. C. Jackson	J. W. Jobling	A. J. Goldforb	" "
1925-27	J. W. Jobling	S. R. Benedict	" "	" "

<i>Date</i>	<i>President</i>	<i>Vice-President</i>	<i>Secretary</i>	<i>Treasurer</i>
1927-29	S. R. Benedict	P. Rous	A. J. Goldforb	
1929-30	P. Rous	D. Marine	"	"
1930-31	P. Rous	D. J. Edwards	"	"
1931-32	D. J. Edwards	A. R. Dochez	"	"
1932-34	A. R. Dochez	E. L. Opie	"	"
1934-36	E. L. Opie	P. E. Smith	"	"
1936-37	P. E. Smith	E. F. DuBois	"	"
1937-39	H. S. Gasser	J. T. Wearn	"	"

SECTIONAL MEETINGS AND MEMBERSHIP

Cleveland, Ohio

Chairman: J. M. Hayman, Jr. Secretary: H. D. Green. Members: 42.

Meetings: Western Reserve University, October 14, 1938

November 11, 1938

December 9, 1938

January 13, 1939

February 10, 1939

March 10, 1939

April 14, 1939

May 12, 1939

District of Columbia

Chairman: C. E. Leese. Secretary: D. B. Jones. Members: 41.

Meetings: National Institute of Health, December 15, 1938

U. S. Public Health Service, March 16, 1939

Wesley Hall, June 1, 1939

Illinois

Chairman: S. Soskin. Secretary: L. A. Crandall, Jr. Members: 134.

Meetings: Northwestern University, November 8, 1938

University of Illinois Medical School, January 24, 1939

University of Chicago, March 7, 1939

Northwestern University, May 23, 1939

Iowa

Chairman: W. R. Ingram. Secretary: T. L. Jahn. Members: 38.

Meetings: State University of Iowa, November 29, 1938

February 2, 1939

May 11, 1939

Minnesota

Chairman: F. H. Scott. Secretary: F. H. Scott. Members: 47.

Meetings: University of Minnesota, November 16, 1938

January 18, 1939

February 15, 1939

April 19, 1939

May 17, 1939

Missouri

Chairman: A. B. Hertzman. Secretary: H. L. White. Members: 57.
 Meetings: St. Louis University Medical School, October 12, 1938
 Washington University Medical School, November 9, 1938
 St. Louis University Medical School, February 8, 1939
 Washington University Medical School, March 8, 1939
 Washington University Medical School, May 10, 1939

New York

Chairman: M. H. Dawson. Secretary: I. Greenwald. Members: 833.
 Meetings: New York Academy of Medicine, October 26, 1938
 College of Physicians and Surgeons, November 23, 1938
 New York University Medical School, January 25, 1939
 Rockefeller Institute, March 22, 1939
 Mount Sinai Hospital, May 24, 1939

Pacific Coast

Chairman: A. W. Meyer. Secretary: C. Weiss. Members: 91.
 Meetings: Stanford University, October 15, 1938
 University of California Medical School, December 7, 1938
 Stanford-Lane Hospital, February 1, 1939
 University of California, May 6, 1939

Peiping, China

Chairman: S. H. Liu. Secretary: C. H. Hu. Members: 29.
 Meetings: Peiping Union Medical College, November 5, 1938
 January 12, 1939
 March 17, 1939

Southern

Chairman: H. S. Mayerson. Secretary: R. Ashman. Members: 41.
 Meetings: Tulane University, November 11, 1938
 December 16, 1938
 January 31, 1939
 May 12, 1939

Southern California

Chairman: C. H. Thienes. Secretary: E. Bogen. Members: 40.
 Meetings: University of Southern California, October 25, 1938
 California Institute of Technology, December 28, 1938
 Zoo Hospital, March 25, 1939
 University of California, Los Angeles, May 22, 1939

Western New York

Chairman: E. F. Adolph. Secretary: H. C. Hodge. Members: 61.
 Meetings: Cornell University, October 29, 1938
 Clifton Springs Sanitarium, December 10, 1938
 University of Rochester, February 18, 1939
 University of Buffalo, May 20, 1939

Wisconsin

Chairman: W. E. Sullivan. Secretary: O. O. Meyer. Members: 37.
 Meeting: University of Wisconsin, May 16, 1939

MEMBERSHIP

Members, March 31, 1938.....	1419		
Elected during year.....	81		
Total		1500	
Resignations	4		
Deaths	8	12	
Total Membership, March 31, 1939.....		1488	
	1929	1939	
Membership.....	986	1488	
Subscriptions, March 31, 1939.....			648

DEATHS OF MEMBERS

The Council records with regret the deaths of the following members:
 Doctors J. J. Abel, D. H. Bergey, M. Brodie, G. E. Burget, W. H. Park,
 A. L. Prince, D. H. Shelling, M. J. Sittenfield, C. R. Stockard, W. C. Thro,
 T. W. Todd and E. B. Wilson.

A. J. GOLDFORB,
Secretary.

TREASURER'S REPORT

April 1, 1938 to April 1, 1939

RECEIPTS

Balance on Hand, April 1, 1938.....	\$ 9,133.42*
Income, 1938-39	
Dues	\$ 5,939.13
Reprints	2,741.69
Excess Space	1,034.34
Cuts	638.80
Changes	27.04
Subscriptions	3,937.14
Back Numbers Sold.....	143.27
Interest (Special Accounts).....	108.25
Miscellaneous	26.63
Total Annual Income	\$14,596.29
Total Cash Available, April 1, 1938-April 1, 1939.....	\$23,729.71

DISBURSEMENTS

Publication Cost of PROCEEDINGS:	
Printing	\$ 7,961.09
Reprints	2,861.35
Cuts	679.40
	\$11,501.84
Administrative Expenses:	
Office Supplies, Postage and Telephone.....	\$ 628.26
Salary	2,161.00
Storage and Insurance.....	43.00
Miscellaneous	150.78
	2,983.04
Total Annual Disbursements.....	\$14,484.88
Cash Balance, April 1, 1939.....	9,244.83*
Total	\$23,729.71

SUMMARY

Income (net)	\$14,596.29
Disbursements (net)	14,484.88
Surplus	\$ 111.41
Accounts Payable—none	
Accounts Receivable—\$932.24	

*Includes Endowment Fund temporarily in Corn Exchange Bank—\$1,811.67.

TREASURER'S REPORT

FUNDS

Endowment Fund

April 1, 1938.....	\$16,630.82	
Interest to April 1, 1939.....	673.86	
		\$17,304.68
Invested in New York Title and Mortgage Co.....	\$ 5,940.00	
Title Guarantee and Trust Co.....	2,000.00	
Lawyers Mortgage Company.....	1,500.00	
Railroad Federal Savings and Loan.....	25.81	
Bowery Savings Bank.....	2,305.71	
Industrial Bonds	3,721.49	
Temporarily in Corn Exchange Bank.....	1,811.67	
		\$17,304.68

Surplus Fund

April 1, 1938.....	\$10,504.27	
Interest to April 1, 1939.....	419.89	
		\$10,924.16
Invested in Title Guarantee and Trust Co.....	\$ 2,868.50	
Railroad Federal Savings and Loan.....	981.03	
Bowery Savings Bank	1,200.44	
Industrial Bonds	5,874.19	
		\$10,924.16

Life Membership Fund

Invested in Railroad Federal Savings and Loan.....	\$ 75.00
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Auditors' Report

We the undersigned have this day examined the Treasurer's report and find it to agree with the books of the Society. We believe that the records of the financial transactions are accurate and in good order.

We concur with the committee of the previous year in the judgment that a certified public accountant should be engaged to make periodic audits of the Treasurer's records for the protection of the Secretary-Treasurer and the members of the Auditing Committee.

(Signed)

JAMES A. SHANNON
AURA E. SEVERINGHAUS

April 24, 1939.

MEMBERS' LIST

HONORARY MEMBERS

Flexner, Simon.....	Rockefeller Institute
Howell, William H.....	Johns Hopkins Univ.
Lee, F. S.....	New York City
Lombard, Warren P.....	Univ. of Michigan
Porter, William.....	Harvard Univ.
Richet, Charles.....	Paris, France
Von Muller, Friedrich.....	Munich, Germany

MEMBERS

Abramson, H. A.....	Coll. Physicians and Surgeons, New York
Abt, Arthur F.....	Northwestern Univ.
Adams, A. Elizabeth.....	Mount Holyoke Coll.
Adams, William E.....	Univ. of Chicago
Addis, Thomas.....	Med., Stanford Univ.
Adolph, E. F.....	Univ. of Rochester Med.
Adolph, W. H.....	Peiping, China
Alexander, Harry L.....	Washington Univ.
Allen, Bennet M.....	Univ. of California, Los Angeles
Allen, Edgar.....	Yale Univ.
Allen, William F.....	Univ. of Oregon
Alles, G. A.....	Los Angeles, California
Alt, Howard L.....	Northwestern Univ. Med.
Althausen, T. L.....	Univ. of California Med.
Altschule, M. D.....	Beth Israel Hospital, Boston
Alvarez, Walter C.....	Mayo Clinic
Alving, A. S.....	Univ. of Chicago
Amberg, Samuel.....	Mayo Clinic
Amberson, W. R.....	Univ. of Maryland Med.
Amoss, Harold L.....	Rockefeller Institute
Anderson, Dorothy H.....	Coll. of Physicians and Surgeons
Anderson, H. H.....	Chicago, Ill.
Anderson, John E.....	Univ. of Minnesota
Anderson, John F.....	E. R. Squibb & Son
Anderson, Rudolph J.....	Yale Univ.
Anderson, William E.....	Springfield, Mass.
Andrews, Edmund.....	Univ. of Chicago
Ansbacher, Stefan.....	Squibb Inst., New Brunswick, N. J.
Anson, Mortimer L.....	Rockefeller Institute, Princeton, N. J.
Antopol, William.....	Beth Israel Hospital, Newark, N. J.
Apperly, Frank L.....	Med. Coll. of Virginia
Armstrong, Charles.....	National Inst. of Health, Washington
Arnold, Lloyd.....	Univ. of Illinois
Aronson, J. D.....	Henry Phipps Institute

Asdell, S. A.....	Cornell Univ.
Asher, Leon.....	Berne, Switzerland
Ashman, Richard.....	Louisiana State Univ. Medical Center
Asmundson, V. S.....	Univ. of California
Atchley, D. W.....	Presbyterian Hospital, New York City
Atkinson, H. V.....	Vermillion, S. D.
Atwell, Wayne J.....	Univ. of Buffalo, N. Y.
Aub, Joseph C.....	Huntington Memorial Hospital, Boston
Auer, John.....	St. Louis Univ.
Austin, J. Harold.....	Univ. of Pennsylvania
Avery, O. T.....	Rockefeller Institute, N. Y. City
Avery, Roy C.....	Vanderbilt Univ.
Aycock, W. L.....	Harvard Med.
B abkin, Boris P.....	McGill Univ.
Bachem, Albert.....	Univ. of Illinois Medical Coll.
Baehr, George.....	Mt. Sinai Hospital, N. Y. City
Bagg, Halsey J.....	Memorial Hospital, N. Y. City
Bahrs, Alice M.....	Portland, Ore.
Bailey, Cameron V.....	N. Y. Post-Graduate Med.
Baitsell, George A.....	Yale Univ.
Baker, Lillian E.....	Rockefeller Institute
Bakwin, Harry.....	N. Y. Univ. and Bellevue Medical Coll.
Baldwin, Francis M.....	Univ. of S. Calif.
Ball, G. H.....	Univ. of California, Los Angeles
Ball, H. A.....	San Diego, Calif.
Balls, A. K.....	U. S. Dept. of Agriculture
Banta, A. M.....	Carnegie Institution, Cold Spring Harbor
Banting, Frederick G.....	Univ. of Toronto
Barach, Alvin L.....	Coll. of Physicians and Surgeons
Barber, W. Howard.....	New York Univ. Med.
Barbour, Henry G.....	Yale Univ.
Barer, Adelaide P.....	State Univ. of Iowa
Barlow, O. W.....	Rensselaer, N. Y.
Barnett, George D.....	Stanford Univ.
Barr, David P.....	Washington Univ.
Barron, E. S. G.....	Univ. of Chicago
Barth, L. G.....	Columbia Univ.
Bartley, S. H.....	Washington Univ.
Bass, Charles.....	Tulane Univ.
Bast, T. H.....	Univ. of Wisconsin
Bates, R. W.....	Carnegie Institution of Washington
Bauer, J. H.....	Rockefeller Institute
Bauman, Louis.....	Presbyterian Hospital, N. Y. City
Baumann, E. J.....	Montefiore Hospital, N. Y. City
Baumberger, J. Percy.....	Stanford Univ.
Bayne-Jones, S.....	Yale Univ.
Bazett, H. C.....	Univ. of Pennsylvania
Bean, John W.....	Univ. of Michigan
Beard, H. H.....	Louisiana State Univ. Medical Center
Beard, J. W.....	Duke University

Beard, P. J.	Stanford Univ.
Beck, Claude S.	Western Reserve Univ.
Becker, E. R.	Iowa State Coll.
Beckman, Harry	Marquette Univ. Med.
Beckwith, T. D.	Univ. of California, Los Angeles
Behre, Jeannette A.	Cornell Univ. Medical Coll.
Belding, David L.	Boston Univ.
Bellamy, Arthur W.	Univ. of California, Los Angeles
Bengtson, Ida A.	National Inst. of Health, Washington
Berg, B. N.	Columbia Univ.
Berg, C. P.	State Univ. of Iowa
Berg, William N.	New York City
Bergeim, Olaf	Univ. of Illinois
Bergmann, Max	Rockefeller Institute
Bernhard, Adolph	Lenox Hill Hospital, N. Y. City
Bernthal, T. G.	Univ. of Michigan
Berry, George P.	Univ. of Rochester Med.
Beutner, R.	Hahnemann Medical College, Philadelphia
Bieter, Raymond N.	Univ. of Minnesota
Bills, C. E.	Mead, Johnson and Co., Evansville, Ind.
Bing, Franklin C.	American Med. Assn., Chicago
Binger, Carl A. L.	Rockefeller Institute, N. Y. City
Birkhaug, Konrad E.	Geophysic Institute, Bergen, Norway
Bishop, George H.	Webster Groves, Mo.
Bishop, Katharine S.	Univ. of California
Blackfan, K. D.	Harvard Med.
Blair, John E.	Hospital for Joint Diseases, N. Y. City
Blake, F. G.	Yale Univ.
Blakeslee, Albert F.	Station of Exp. Evolution, Cold Spring Harbor, N. Y.
Blalock, Alfred	Vanderbilt Univ. Med.
Blatherwick, Norman R.	Metropolitan Life Insurance Co., N. Y. City
Blau, Nathan F.	Cornell Univ. Medical Coll.
Blinks, L. R.	Stanford Univ.
Bliss, Sidney	Tulane Univ.
Bloch, Robert G.	Univ. of Chicago
Block, Richard J.	N. Y. State Psychiatric Institute
Bloom, William	Univ. of Chicago
Bloomfield, A. L.	Stanford Univ. Med.
Bloor, W. R.	Univ. of Rochester
Blount, R. F.	Univ. of Minnesota
Blum, Harold F.	Washington, D. C.
Blumberg, Harold	Johns Hopkins University
Blumgart, H. L.	Beth Israel Hospital, Boston
Bock, Joseph C.	Marquette Univ.
Bodansky, A.	Hospital for Joint Diseases, N. Y. City
Bodansky, Meyer	John Sealy Hospital, Galveston, Texas
Bodansky, Oscar	New York Univ.
Bodine, J. H.	State Univ. of Iowa
Bodo, Richard	N. Y. Univ. and Bellevue Medical Coll.
Bogen, Emil	Olive View, Calif.
Boissevain, Charles H.	Colorado College

Bollman, Jesse L.....	Mayo Clinic
Boor, Alden K.....	Univ. of Chicago
Boothby, Walter M.....	Kahler Hospital, Rochester, Minn.
Boots, Ralph H.....	Presbyterian Hospital, N. Y. City
Borsook, Henry.....	California Institute of Technology
Bowen, B. D.....	Buffalo General Hospital
Boyd, Eldon M.....	Queens University, Canada
Boyd, Theo. E.....	Loyola Univ.
Boyden, E. A.....	Univ. of Minnesota Med.
Bozler, Emil.....	Ohio State Univ.
Bradford, William L.....	Univ. of Rochester
Bradley, H. C.....	Univ. of Wisconsin
Brand, Erwin.....	N. Y. State Psychiatric Institute
Branham, Sara E.....	National Inst. of Health, Washington
Brewer, Robert K.....	Syracuse Univ.
Briggs, A. P.....	Univ. of Georgia
Bronfenbrenner, J.....	Washington Univ.
Bronk, D. W.....	Univ. of Pennsylvania
Brooks, Clyde.....	Louisiana State Univ. Medical Center
Brooks, Matilda M.....	Univ. of California
Brooks, S. C.....	Univ. of California
Broun, G. O.....	St. Louis Univ. Med.
Brown, J. Howard.....	Johns Hopkins Univ.
Brown, John B.....	Ohio State Univ.
Brown, L. A.....	Transylvania Coll., Lexington, Ky.
Brown, Rachel.....	N. Y. State Dept. of Health
Brown, Wade H.....	Rockefeller Institute, Princeton
Browne, J. S. L.....	Royal Victoria Hosp., Montreal
Bruger, Maurice.....	N. Y. Post-Graduate Med.
Brunschwig, Alexander.....	Univ. of Chicago
Buchanan, Robert E.....	Iowa State Coll.
Buchbinder, W. C.....	Michael Reese Hospital, Chicago
Buell, Mary V.....	Johns Hopkins Univ.
Bulger, H. A.....	Washington Univ.
Bullowa, J. G. M.....	Harlem Hospital, N. Y. City
Bunting, C. H.....	Univ. of Wisconsin
Burch, George E.....	Tulane University
Burch, John C.....	Vanderbilt Univ. Med.
Burdon, Kenneth L.....	Louisiana State Univ.
Burky, Earl L.....	Johns Hopkins Hospital
Burns, Robert K., Jr.....	Univ. of Rochester
Burr, George O.....	Univ. of Minnesota
Burrows, M. T.....	Pasadena, Calif.
Butcher, E. O.....	Hamilton Coll.
Butt, E. M.....	Univ. of Southern California
Butts, Joseph S.....	Univ. of Southern California
Byerly, T. C.....	U. S. Animal Exp. Farm, Beltsville, Md.
Byrne, Joseph.....	Fordham Univ.

Calkins, Gary N.....Columbia Univ.
Cameron A. T.....Univ. of Manitoba

Cannan, Robert K.	N. Y. Univ. and Bellevue Medical Coll.
Cannon, Paul R.	Univ. of Chicago
Cannon, Walter B.	Harvard Med.
Cantarow, Abraham	Jefferson Medical Coll.
Carey, E. J.	Marquette Univ.
Carlson, A. J.	Univ. of Chicago
Carmichael, E. B.	Univ. of Alabama Med.
Carmichael, L.	Tufts College
Carruthers, A.	Birmingham, England
Casey, Albert E.	Louisiana State Univ.
Cash, James R.	Univ. of Virginia
Casida, L. E.	Univ. of Wisconsin
Cattell, McKeen	Cornell Univ. Medical Coll.
Caulfeild, A. H. W.	Univ. of Toronto
Cecil, R. L.	Cornell Univ. Medical Coll.
Cerecedo, L. R.	Fordham Univ.
Chace, Arthur F.	N. Y. Post-Graduate Medical Coll.
Chaikoff, I. L.	Univ. of California
Chambers, Robert	New York Univ.
Chambers, William H.	Cornell Univ. Medical Coll.
Chang, Hsi Chun	Peiping Union Medical Coll.
Chang, Hsiao-Chien	Hunan, China
Chargaff, Erwin	Coll. of Physicians and Surgeons
Charipper, H. A.	New York Univ.
Cheer, S. N.	W. China Union Univ.
Chen, Graham M.	Univ. of Chicago
Chen, K. K.	Eli Lilly and Co., Indianapolis
Chen, T. T.	Peiping Union Medical Coll.
Cheney, R. H.	Long Island Univ.
Chidester, F. E.	Newark Valley, N. Y.
Child, C. M.	Stanford Univ.
Chittenden, R. H.	Yale Univ.
Chouke, K. S.	Univ. of Colorado
Chow, B. F.	Squibb Inst., New Brunswick, N. J.
Christensen, K.	St. Louis Univ.
Christian, Henry A.	Peter Bent Brigham Hospital
Christman, Adam A.	Univ. of Michigan
Chu, F. T.	Peiping Union Medical Coll.
Clark, A. J.	University of Edinburgh, Scotland
Clark, Ada R.	Coll. of Physicians and Surgeons
Clark, Guy W.	Lederle Lab., Pearl River, N. Y.
Clark, P. F.	Univ. of Wisconsin
Clarke, Hans T.	Coll. of Physicians and Surgeons
Claude, A.	Rockefeller Inst.
Clausen, H. J.	Univ. of Colorado Med.
Claussen, S. W.	Strong Memorial Hospital, Rochester, N. Y.
Clawson, Benjamin J.	Univ. of Minnesota
Clifton, Charles E.	Stanford Univ.
Clowes, G. H. A.	Eli Lilly and Co., Indianapolis, Indiana
Coca, A. F.	Oradell, N. J.
Coggeshall, L. T.	Rockefeller Foundation

Coghill, G. E.	Gainesville, Fla.
Cohen, Barnett	Johns Hopkins Med.
Cohen, Martin	N. Y. Post-Graduate Med.
Cohen, Milton B.	St. Alexis Hospital, Cleveland
Cohn, A. E.	Rockefeller Institute, N. Y. City
Cohn, Isidore	New Orleans, La.
Cole, Arthur G.	Univ. of Illinois Med.
Cole, E. C.	Williams Coll.
Cole, Harold H.	Univ. of California, Davis
Cole, L. J.	Univ. of Wisconsin
Cole, Rufus I.	Rockefeller Institute, N. Y. City
Cole, Warren H.	Univ. of Illinois Med.
Cole, William H.	Rutgers Univ.
Collens, William S.	Brooklyn, N. Y.
Collett, Mary E.	Western Reserve Univ.
Collier, William D.	St. Elizabeth's Hospital, Youngstown, O.
Collins, D. A.	Temple Univ.
Collip, J. B.	McGill Univ.
Compere, E. L.	Univ. of Chicago
Conklin, E. G.	Princeton Univ.
Connor, Charles L.	Univ. of California Med.
Cook, Donald H.	School of Tropical Medicine, San Juan, P. R.
Cooke, J. V.	Washington Univ.
Coombs, Helen C.	N. Y. Homeopathic Medical Coll.
Cooper, Frank B.	Western Pennsylvania Hosp., Pittsburgh
Cope, O. M.	N. Y. Homeopathic Med.
Copenhaver, W. M.	Columbia Univ.
Corey, E. L.	Univ. of Va.
Cori, Carl F.	Washington Univ.
Corley, Ralph C.	Purdue Univ.
Corner, George W.	Univ. of Rochester
Corper, H. J.	National Jewish Hospital, Denver, Colo.
Co Tui	N. Y. Univ. Medical Coll.
Coulter, Calvin B.	Columbia Univ.
Cowdry, E. V.	Washington Univ.
Cowgill, George R.	Yale Univ.
Cowie, D. M.	Univ. of Michigan
Cox, Herald R.	U. S. Public Health Inst., Hamilton, Mont.
Cox, Warren M., Jr.	Mead Johnson Co.
Craig, C. F.	Tulane Univ.
Cram, Eloise B.	Nat. Inst. of Health, Washington
Crampton, C. Ward	N. Y. Post-Graduate Med.
Crandall, L. A., Jr.	Northwestern Univ.
Crile, George W.	Western Reserve Univ.
Crittenden, Phoebe J.	George Washington Univ.
Crohn, Burrill B.	Mt. Sinai Hospital, N. Y. City
Csonka, F. A.	U. S. Dept. of Agriculture, Washington, D. C.
Cullen, Glenn E.	Children's Hospital, Cincinnati, O.
Cummins, Harold	Tulane Univ.
Cunningham, Bert	Duke Univ.
Cunningham, R. S.	Albany Med. Coll.

Curtis, G. M.....	Ohio State Univ.
Curtis, Maynie R.....	Columbia Univ.
Cushing, Harvey W.....	Yale Univ.
Cutler, Elliott C.....	Peter Bent Brigham Hospital, Boston
D ack, Gail M.....	Univ. of Chicago
Dakin, H. D.....	Ossining, N. Y.
Dalldorf, Gilbert.....	Grasslands Hosp., Valhalla, N. Y.
D'Amour, F. E.....	Univ. of Denver
Danforth, Charles H.....	Stanford Univ.
Daniel, J. Frank.....	Univ. of California
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